Resuscitation
journal homepage: www.elsevier.com/locate/resuscitation

Clinical paper

Protein S100B as a reliable tool for early prognostication after cardiac arrest

Nicolas Deye[a,b,*,1], *Philippe Nguyen*[a,1], *Nicolas Vodovar*[b], *Malha Sadoune*[b], *Corinne Collet*[b,c], *Sebastian Voicu*[a], *Isabelle Malissin*[a], *Etienne Gayat*[b,d], *Jeanne-Lise Samuel*[b], *Claude Delcayre*[b], *Jean-Marie Launay*[b,c], *Alain Cohen-Solal*[b,e], *Bruno Mégarbane*[a,f], *Alexandre Mebazaa*[b,d]

[a] *Medical ICU, Lariboisiere University Hospital, APHP, Paris, France*
[b] *Inserm UMR-S 942, Lariboisiere Hospital, Paris, France*
[c] *Biochemical Laboratory, Lariboisiere University Hospital, APHP, Paris, France*
[d] *Surgical ICU, Lariboisiere University Hospital, Paris, France*
[e] *Cardiology Department, Lariboisiere University Hospital, Paris, France*
[f] *Inserm U 1140, Lariboisiere Hospital, Paris, France*

Abstract

Purpose: Early and reliable prognostication after cardiac arrest (CA) remains crucial. We hypothesized that protein-S100B (PS100B) could predict more accurately outcome in the early phase of CA compared with other current biomarkers.

Methods: This prospective single-center study included 330 adult comatose non-traumatic successfully resuscitated CA patients, treated with targeted temperature management but not extra-corporeal life support. Lactate, pH, creatinine, NSE, and PS100B were sampled in ICU early after return of spontaneous circulation (ROSC) corresponding to admission (Adm). Serial measurements were also performed at H24 and H48. PS100B was the sole biomarker blinded to physicians.

Measurements and main results: The median delay between ROSC and first PS100B sampling was 220 min. At admission, all biomarkers were significantly associated with good outcome (CPC1−2; 109 patients) at 3-month follow-up ($P \leq 0.001$, except for NSE: $P = 0.03$). PS100B-Adm showed the best AUC of ROC curves for outcome prediction at 3-month (AUC 0.83 [95%-CI: 0.78−0.88]), compared with other biomarkers ($P < 0.0001$), while AUC for lactate-Adm was higher than for NSE-Adm. AUC for PS100B-H24 was significantly higher than for other biomarkers except NSE-H24 ($P \leq 0.0001$), while AUC for NSE-H24 was higher than for lactate-H24 and pH-H24. AUCs for PS100-H48 and NSE-H48 were significantly higher than for all other biomarkers ($P < 0.001$). Compared to patients with decreased PS100B values over time, an increasing PS100B value between admission and H24 was significantly associated with poor outcome at 3 months ($P = 0.001$). No-flow, initial non-shockable rhythm, PS100B-Adm, lactate-Adm, pH-Adm, clinical seizures, and absence of therapeutic hypothermia were independent predictors associated with poor outcome at 3-month in multivariate analysis. Net-Reclassification-Index was 70%, 64%, and 81% when PS100B-Adm was added to the clinical model, to clinical model with NSE-Adm, and to clinical model with standard biological parameters, respectively.

Conclusions: Early PS100B compared with other biomarkers was independently correlated with outcome after CA, with an interesting added value.

Keywords: Heart arrest, Prognostication, Outcome, Protein S100B, NSE, Biomarker

* Corresponding author at: Réanimation Médicale Toxicologique, Hôpital Universitaire Lariboisière, Inserm U942, Assistance Publique des Hôpitaux de Paris, 2 rue Ambroise Paré, 75010, Paris, France.
E-mail addresses: n.deye@orange.fr, nicolas.deye@aphp.fr (N. Deye).
[1] These authors contributed equally to this work and are considered as 2 first co-authors.

https://doi.org/10.1016/j.resuscitation.2020.08.010
Available online xxx

Please cite this article in press as: N. Deye, et al., Protein S100B as a reliable tool for early prognostication after cardiac arrest, Resuscitation (2020), https://doi.org/10.1016/j.resuscitation.2020.08.010

Introduction

Favourable outcome after cardiac arrest (CA) remains poor.[1,2] Early and reliable prognostication in CA patients seems of major importance. It could avoid futile treatment in patients with low chance of good outcome and could help clinicians to maximize treatment in patients who have a high likelihood of good outcome. However, guidelines emphasize that prognostication needs to be delayed, especially when targeted temperature management (TTM) is applied, and that decisions to limit care should be supported by a multimodal approach including clinical, biological, electrophysiological, and/or imaging parameters.[2,3]

The usefulness of biomarkers to help clinicians in optimizing outcome prediction after CA is described mainly for Neuron Specific Enolase (NSE).[2–8] The initial NSE threshold validated in 2006 did not remain 100%-predictive after a broader evaluation in TTM-treated patients,[2,4,5] while NSE accuracy seemed better at a delayed phase after return of spontaneous circulation (ROSC).[3,4,7–9] Other biomarkers, such as lactate, pH, and creatinine levels on admission, have also been proposed to early predict outcome after CA.[10–13] However, specificity of these biomarkers is not 100%, leading to difficulties in assessing their precise cut-off values and predictive accuracy.[4,14] S100B protein (PS100B) is also presumed to be an interesting prognostication tool besides NSE.[3–8,14–22] S100 protein is an intracellular dimeric protein with at least 4 sub-types, with S100A1B and S100BB being presently measured by usual tests.[18] Besides S100A1 protein, S100B protein (PS100B) is normally found in astroglial and Schwann cells, and in neuroctodermal tumoral cells in pathological context, while small amount of PS100 was also found in adipocytes, muscles and chondrocytes. However, the large sub-TTM report by Stammet et al. showed that PS100B could be better than NSE at H24 to predict poor outcome, but the added information was limited in all prognosticating models with or without NSE.[22,23] However, biomarkers were here only sampled 24 h after ROSC and thereafter. Therefore, NSE contrary to PS100B is preferred in international guidelines as a validated and useful biomarker since it is superior at H48 and H72.[2,3]

Considering the relative short half-life of PS100B compared to NSE, we hypothesized that PS100B could more accurately predict the outcome of CA patients in the early phase after ROSC compared to biomarkers such as lactate, pH, creatinine, and NSE. The aim of the present study was to evaluate the usefulness of early PS100B sampling for prognostication in a large cohort of successfully resuscitated CA patients.

Methods

This prospective single-center study was carried out between March 2010 and May 2016 in the medical ICU of a university hospital (Lariboisiere Hospital, Assistance Publique des Hôpitaux de Paris, France). The Ethics Committee of our institution approved the study (Institutional Review Board of Paris North Hospital: CERB GHU Nord, N°00006477). All surviving patients hospitalized for CA -or their next of kin if necessary- gave their written informed consent. The study was declared at National Clinical Trial (NCT01374880).

Aims

Primary endpoint was to assess PS100B performance in discriminating patients with good outcome versus those with poor outcome at 3-month follow-up as soon as the early phase after CA (i.e. as early as possible and within the first 24 h after ROSC). PS100B was compared to other biomarkers (lactate, pH, creatinine, and NSE) in its ability to correctly discriminate outcome.

Secondary endpoints were: 1/ to evaluate PS100B performance in the early phase after CA in discriminating patients with good outcome versus those with poor outcome at hospital discharge; 2/ to evaluate PS100B performance according to the timeframe serum sampling after ROSC versus other biomarkers in its ability to discriminate patients with good outcome versus those with poor outcome (i.e. when sampled within the first 72 h after ROSC).

Patient selection

Were included all consecutive comatose adult patients ≥18 years old, suffering from non-traumatic and successfully resuscitated CA (out-of-hospital or in-hospital CA: OH/IHCA), with sustained ROSC (defined as possibility to maintain ROSC with palpable pulse for >20 min), hospitalized in ICU and TTM-treated. Conscious patients, with unsustained ROSC, refractory CA or cardiogenic shock after CA necessitating an Extra-Corporeal Life Support (ECLS) were excluded. Patients experiencing a do-not-resuscitate order and patients without any PS100B values measured during the ICU course were excluded from this study.

Protocol

The protocol applied for all CA patients has been previously described and was in accordance with international guidelines.[2,3,24] Particularly, TTM was initiated as soon as possible for all patients regardless of the initial cardiac rhythm and ranged from 32 to 36 °C for 24 h. Prognostication and withdrawal of life sustaining therapies, if necessary, were performed according to international guidelines.[2,3]

Outcome assessment and data collection

Data collection followed the Utstein style recommendations.[25] Outcome was evaluated using the Cerebral Performance Category (CPC) scoring, CPC 1–2 representing a good outcome whereas CPC 3–4–5 a poor outcome. CPC was systematically collected at hospital discharge and 3 months after CA, during a face-to-face appointment or by phone, by an independent researcher unblinded to patient medical history.

Blood samples were obtained in ICU for all patients as early as possible after ROSC, and hospital admission for OHCA (corresponding to admission: Adm.), at day 1 (H24: corresponding to the day occurring between 6h00 AM the day following ROSC and the following 24 h), and at day 2 (H48: corresponding to the following 24 h after H24). Two serial blood samples were used to evaluate biomarker variations within the first 3 days after CA. Physicians in charge of the patients were all blinded to PS100B measurements, but not to other biomarkers. PS100B and NSE markers from serum were measured by the sandwich electro-chemi-luminescent immuno-assay (ECLIA) methods using a Cobas E601 analyzer (Roche Diagnostics, Meylan, France), which is similar to those published in largest studies.[8,22]

Statistics

Results are expressed as median (IQR 25–75), unless expressed otherwise. Non-parametric tests were used considering the non-Gaussian distribution of many parameters. Spearman tests for

Please cite this article in press as: N. Deye, et al., Protein S100B as a reliable tool for early prognostication after cardiac arrest, Resuscitation (2020), https://doi.org/10.1016/j.resuscitation.2020.08.010

correlations or Wilcoxon tests for serial measurements were used to compare quantitative values. Kruskal-Wallis and Mann-Whitney tests with Bonferroni correction for multiple comparisons were used to compare quantitative and qualitative parameters. Comparisons for qualitative values were performed using chi-2 and the exact Fisher tests. To assess the biomarker ability to correctly evaluate its prognostic value in terms of good outcome predictions, receiver operating characteristic (ROC) curves with deriving sensitivities, specificities, positive and negative predictive values, associated to different cut-off values were performed. ROC curves and Area Under the Curve (AUC) were compared using the Delong's test. These calculations were performed considering each parameter at admission (Adm: primary endpoint), H24 and H48 on its own, as well as changes over admission to H48. PS100B values at admission were compared versus other major prognostic clinical parameters and biomarkers using univariate and multivariate analyses with backward elimination for multiple linear regressions. Main significant clinical parameters and biological values at admission were used in the multivariate analysis using logistic regression to evaluate early predictors independently associated with good outcome at discharge. The Net Reclassification Index (NRI) was used to assess biomarkers added value in the final model. P-values were considered as significant when <0.01. All statistics were performed using XL-stat Biomed (version 2018.3, Addinsoft, Microsoft, USA), except the NRI performed using R statistical software version 3.1.1 (The "R" Foundation for Statistical Computing, Vienna, Austria).

Results

Among 370 CA without ECLS implementation, 351 patients without exclusion criteria were included, of which 21 were lost to 3-month follow-up (Supplemental Fig. S1). Patients' general characteristics are described in Table 1 and Supplemental Table S1. The delay between CA and the first blood sample measuring NSE and PS100B (Admission) was 245 min [180−338], the delay between ROSC and the first sample being 220 min [155−316]. The delay between ROSC and the second sample (H24) was 23.5 h [15.3−36.0], while it was 48.1 h [39.2−61.2] between ROSC and the third sample (H48).

Biomarker values within the first 3 days are described in Table 2. At admission, all biomarkers were significantly associated with good outcome at 3-month ($P \leq 0.001$), P-value for NSE being 0.03. At H24, all biomarkers except pH were significantly associated with good outcome at hospital discharge ($P < 0.0001$). At H48, PS100B, NSE, and creatinine values were significantly associated with good outcome at hospital discharge ($P \leq 0.004$), but not pH and lactate. Results were similar at hospital discharge (Supplemental Table S2). PS100B and NSE values according to CPC at 3-month are detailed in Fig. 1 and Supplemental Table S3.

Biomarker ROC curves according to the day of sampling are depicted in Fig. 2 and Supplemental Table S4. At admission, best AUC for prediction of outcome was observed for PS100B-Adm compared with all other biomarkers ($P < 0.0001$), AUC for lactate-Adm being also higher than for NSE-Adm (Supplemental Fig. S2a). At H24, AUC for PS100B-H24 was significantly higher than for all other biomarkers except NSE-H24 ($P \leq 0.0001$, Supplemental Fig. S2b), while AUC for NSE-H24 was higher than AUCs for lactate-H24 and pH-H24. At H48, best AUCs were observed for both PS100B-H48 and NSE-H48 versus all other biomarkers for outcome prediction ($P < 0.001$, Supplemental Fig. S2c). No differences were observed between all PS100B AUCs from admission to H48, whereas AUC for NSE-Adm was significantly lower than AUCs for NSE-H24 and NSE-H48 ($P < 0.0001$, Supplemental Fig. S3).

PS100B serial measurements are described in Table 3. PS100B values significantly decreased over time ($P < 0.0001$). An increasing value between admission and H24 was significantly associated with poor outcome compared to patients with decreased PS100B values over time ($P = 0.001$, only 1 patient with good outcome experiencing an increasing PS100B value). Comparable results were observed for PS100B differences between admission and H48 measurements but not between H24 and H48.

Biomarkers sensitivity and specificity according to the maximum accuracy at all time-points are detailed in Table 4. A PS100B-Adm threshold of 3.78 μg/L correctly classified all patients except one as experiencing poor outcome with a 99%-specificity (95%-CI [94−99]) and a positive predictive value of 98% (1 false positive; Supplemental Fig. S4, Tables S5 and S6).

In multivariate analysis, early predictors independently associated with poor outcome at hospital discharge were: no-flow duration, PS100B-Adm, pH-Adm, and lactate-Adm values, initial non-shockable rhythm, clinical seizures, and absence of therapeutic hypothermia (Supplemental Table S7, AUC of the model: 0.91 [95%-CI: 0.86−0.95]). NRI was 70%, 64%, and 81% when PS100B-Adm was added to the clinical model alone, to the clinical model with NSE-Adm, and to the model including clinical with standard biological parameters, respectively (Table 4 and Supplemental Table S8).

Discussion

Results can be summarized as follows: 1/ Initial PS100B after admission was significantly associated with good outcome at hospital discharge and 3-month after CA; 2/ PS100B-Adm. was the most accurate biomarker to correctly predict good outcome as evaluated by ROCs analyses, compared with lactate, creatinine, pH, and NSE; 3/ An increasing PS100B value from admission to H24 was significantly associated with poor outcome (1 false negative); 4/ A PS100B-Adm. threshold of 3.78 μg/L correctly classified all patients except one as belonging to poor outcome group; 5/ In multivariate analysis, PS100B-Adm. was associated with poor outcome at 3-month, besides no-flow duration, initial non-shockable rhythm, lactate-Adm. and pH-Adm. values, presence of clinical seizures, and absence of therapeutic hypothermia; 6/ NRI was 70%, 64%, and 81% when PS100B-Adm was added to the clinical model alone, to the clinical model with NSE-Adm, and to the model including clinical with standard biological parameters, respectively; 7/ PS100B and NSE were highly discriminative at H24 and H48 to correctly predict good outcome.

Prognostication after CA using PS100B/NSE

In the meta-analysis describing biomarkers and outcome before TTM era, biomarkers values associated with a 0% false positive rate (FPR) varied considerably within studies with a very low level of evidence according to the timing of sampling.[26] After TTM implementation, other studies were reported.[4] Two studies observed PS100B values of 0.18 μg/L and 0.21 μg/L at H24 as predicting poor outcome in 100% of cases.[7,27] One of these studies showed a sensitivity of 65%, FPR being 4%, and the other that PS100B measured 24 h after ROSC was the best predictor of poor outcome (sensitivity: 87%, specificity:

Please cite this article in press as: N. Deye, et al., Protein S100B as a reliable tool for early prognostication after cardiac arrest, Resuscitation (2020), https://doi.org/10.1016/j.resuscitation.2020.08.010

Table 1 – Patient general characteristics according to outcome at 3 months.

Characteristic	Overall (N = 330)	Good outcome (N = 109)	Poor outcome (N = 221)	P
Age, yr	61 [49–72]	55 [45–67]	63 [52–76]	0.0002
Male sex, no. (%)	230 (70)	84 (77.1)	146 (66.1)	0.043
Body Mass Index, kg/m^2	26 [23–29]	25 [24–29]	26 [23–30]	0.54
Previous hypertension, no. (%)	126 (38.1)	36 (33.0)	90 (40.7)	0.23
Previous coronary disease	60 (18.1)	13 (11.9)	47 (21.3)	0.034
Previous chronic heart failure	29 (8.8)	6 (5.5)	23 (10.4)	0.15
Diabetes, no. (%)	72 (21.8)	15 (13.8)	57 (25.8)	0.01
Chronic renal failure, no. (%)	25 (7.6)	1 (0.9)	24 (10.9)	0.001
Location of arrest: in-hospital, no. (%)	79 (23.9)	24 (22.0)	55 (24.9)	0.59
Location of arrest: out-of-hospital, no. (%):	251 (76.1)	85 (78.0)	166 (75.1)	
Home, no. (%)	127 (50.6)	36 (48.0)	91 (54.8)	
Public place, no. (%)	124 (49.4)	49 (52.0)	75 (45.2)	
Bystander, no (%)	312 (94.3)	103 (94.5)	209 (94.6)	0.99
Bystander CPR, no. (%)	226 (68.3)	87 (79.8)	139 (62.9)	0.002
Initial documented rhythm, no. (%)				<0.0001
Shockable rhythm: VF / pulseless VT, no. (%)	125 (37.8)	80 (73.4)	45 (20.4)	
Non-shockable rhythm: asystole / PEA, no. (%)	206 (62.2)	29 (26.6)	176 (79.6)	
No-flow, min[a]	2 [0–8]	1 [0–4]	4 [0–10]	0.0001
Low-flow, min[a]	17 [10–27]	13 [7–23]	19 [12–28]	0.0005
Adrenaline bolus, mg (total IV, N = 243)	2 [0–4]	0 [0–2]	2 [1–4]	<0.0001
Initial catecholamine perfusion, mg/h (N = 162)	0.5 [0–1.5]	0 [0–1]	1 [0–2]	<0.0001
SAPS II	69 [56–83]	63 [53–73]	73 [59–86]	<0.0001
Cardiac cause responsible for or participating to CA, including APE and PE, no (%)[b]	190 (57.4)	91 (83.5)	99 (44.8)	<0.0001
CA related to acute coronary occlusion, no (%)[b]	115/220 (52.3)	65/90 (72.2)	50/130 (38.5)	<0.0001
PCI with stenting, no (%)[b]	82/220 (37.3)	49/90 (54.4)	33/130 (25.4)	<0.0001
Post-CA shock, no. (%)	234 (70.7)	70 (64.2)	164 (74.2)	0.092
Clinical seizures, no. (%)	62 (18.7)	10 (9.2)	52 (23.5)	0.002
Acute hepatic injury and failure, no. (%)	103 (31.1)	27 (24.8)	76 (34.4)	0.078
DIC, no. (%)	64 (19.3)	16 (14.7)	48 (21.7)	0.14
Renal replacement, no. (%)	50 (15.1)	9 (8.3)	41 (18.6)	0.014
32–34 °C TTM, no (%)[c]	266 (80.4)	102 (93.6)	164 (74.2)	<0.0001
Mechanical ventilation duration[d], days	6 [3–10]	5 [3–10]	6 [3–10]	0.75
Decision to limit treatment(s), no. (%)	149 (45.0)	0	149 (67.4)	<0.0001
Delay to first treatment limitation decision, days	4 [2–7]	0	4 [2–7]	0.10
Duration of hospitalization in ICU, days	7 [4–13]	9 [6–16]	7 [4–12]	0.001
In-hospital length of stay, days	10 [5–22]	21 [14–31]	7 [4–13]	<0.0001

Abbreviation: CPR: cardio-pulmonary resuscitation; IV: intra-venous; Low-flow time: delay between the first CPR and the return of spontaneous circulation (ROSC); No-flow time: delay between collapse (or time for emergency call in non-witnessed cardiac arrests) and the first CPR; PEA: pulseless electrical activity; VF: ventricular fibrillation; VT: ventricular tachycardia; SAPS II: simplified acute physiologic score II; CA: cardiac arrest, DIC: disseminated intravascular coagulation; PCI: percutaneous coronary intervention; TTM: targeted temperature management.

[a] Time between collapse to ROSC was 22 min [14–33].

[b] Coronary angiogram was performed in 220/330 patients (67%), brain and chest CT-scans in 194/330 (59%) and 132/330 (40%), respectively.

[c] All patients were TTM-treated between 32 °C and 36 °C.

[d] All patients were initially intubated and mechanically ventilated after cardiac arrest. Good outcome depicts CPC 1 and 2 at hospital discharge, and poor outcome CPC 3 to 5. Survival and good outcome were observed in 45% and 32% at ICU discharge, in 44% and 36% at hospital discharge, and in 39% and 33% at 3 months after CA, respectively.

100%). Another study described a threshold of 0.3 μg/L at H48 associated with outcome with a 21%-sensitivity and 0% FPR.[28] None of these 3 studies evaluated PS100B in large enough populations.

The largest study sampling PS100B after 24 h post-CA showed that PS100B could be better than NSE at H24 to predict outcome, but the added information was limited in all prognosticating models with or without NSE.[22] Interestingly, AUC for PS100B-H24 observed in our paper and this study were globally similar. However, early prognostication was herein not evaluated, biomarkers not being sampled immediately after admission. Our study affirms in another large cohort that PS100B adds a significant value regarding prognostication, while NSE is robust to accurately predict outcome at a delayed phase after CA, as previously suggested.[4,6,8,19,22]

By contrast to PS100B keeping interesting NRI values from admission to H48, NSE's NRI increases progressively after H24 where its value contributes to neuroprognostication, these biomarkers describing two aspects of brain injury (i.e. glial versus neuronal cells). According to PS100B high specificity/low sensitivity on admission and the validated NSE value at H24-H48, we could use PS100B from admission to H48 and NSE samples from H24 to H72 to better refine the neuroprognostication process. However, this suggestion needs to be adapted in each centre and to strictly follow prognostication

Please cite this article in press as: N. Deye, et al., Protein S100B as a reliable tool for early prognostication after cardiac arrest, Resuscitation (2020), https://doi.org/10.1016/j.resuscitation.2020.08.010

Table 2 – Biomarker values within the first 3 days after cardiac arrest according to outcome at 3-month follow-up.

Biomarker	Overall (N = 330)	Good outcome (N = 109)	Poor outcome (N = 221)	P
Lactate Adm, mMol/L	6.5 [3.0–10.1]	3.8 [2.3–6.7]	8.0 [4.7–11.6]	<0.0001
pH Adm	7.21 [7.11–7.31]	7.26 [7.17–7.33]	7.20 [7.08–7.29]	0.001
Creatinine Adm, µMol/L	105 [84–148]	93 [77–113]	111 [89–165]	<0.0001
PS100B Adm, µg/L	0.90 [0.30–2.16]	0.28 [0.15–0.52]	1.38 [0.64–3.41]	<0.0001
NSE Adm, µg/L	27 [20–39]	24 [19–35]	29 [21–40]	0.03
Lactate H24, mMol/L	2.5 [1.5–4.8]	1.9 [1.3–3.1]	2.9 [1.8–5.8]	<0.0001
pH H24	7.30 [7.23–7.39]	7.32 [7.26–7.38]	7.30 [7.22–7.39]	0.16
Creatinine H24, µMol/L	83 [62–143]	69 [56–92]	107 [66–182]	<0.0001
PS100B H24, µg/L	0.26 [0.12–0.84]	0.12 [0.08–0.20]	0.55 [0.19–1.44]	<0.0001
NSE H24, µg/L	33 [22–63]	24 [18–34]	42 [26–91]	<0.0001
Lactate H48, mMol/L	1.8 [1.3–2.8]	1.8 [1.2–2.7]	1.8 [1.3–2.9]	0.16
pH H48	7.36 [7.30–7.44]	7.36 [7.33–7.41]	7.36 [7.28–7.45]	0.79
Creatinine H48, µMol/L	81 [61–153]	75 [60–96]	96 [63–195]	0.004
PS100B H48, µg/L	0.17 [0.10–0.57]	0.10 [0.08–0.14]	0.36 [0.14–0.96]	<0.0001
NSE H48, µg/L	30 [19–84]	21 [17–28]	58 [25–144]	<0.0001

Abbreviation: Adm: day of initial sampling after return of spontaneous circulation, i.e. after admission; H24 corresponds to the following 24 h after the day of initial sampling, and H48 to the following 24 h after H24; NSE: Neuron Specific Enolase; PS100B: protein S100B.
The total number of samples for PS100B and NSE were on admission: 262 and 256, at H24: 243 and 237, at H48: 224 and 214, respectively. The number of PS100B and NSE samples per patient during the first 72 h after cardiac arrest were 2 [2–3] and 3 [1–3], respectively. Good outcome depicts CPC 1 and 2 at M3 after cardiac arrest, and poor outcome CPC 3–5 at M3-follow-up.

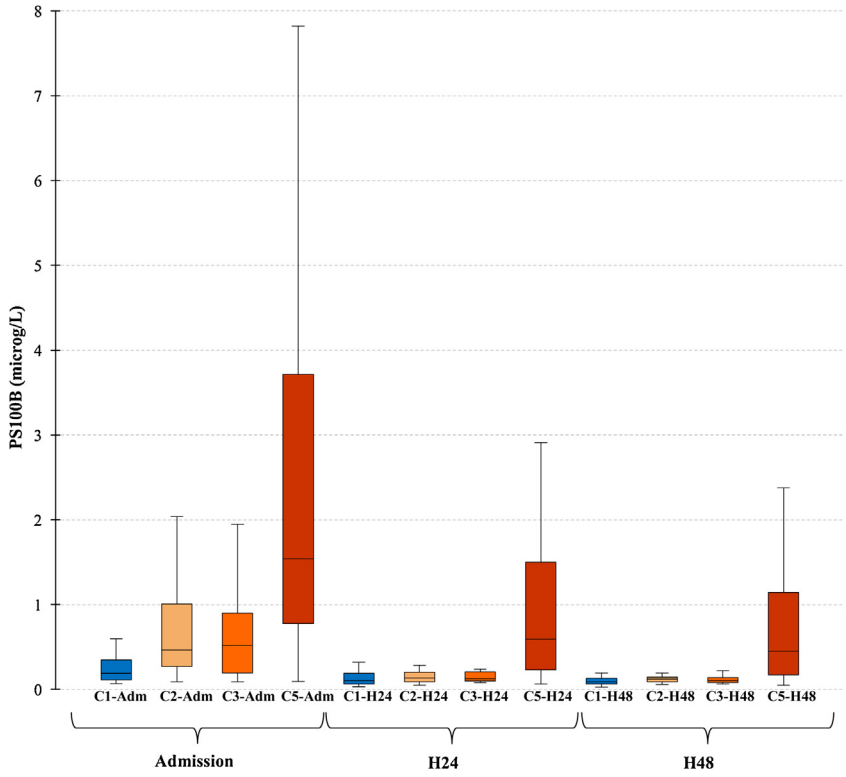

Fig. 1 – Protein S100 time course according to CPC assessment at 3 months after cardiac arrest (N = 330).
Box plots represent PS100B sampling over the first 3 days after CA. Data are presented as median, quartiles 1 and 3, lower and upper fence.
Legend (x axis) = Adm: day of sampling after admission in ICU, H24 and H48 correspond to the day of sampling during the following 24 h and 48 h. « C » denotes CPC assessment, ranging from CPC-1 (no sequellae) to CPC-5 (death or brain death). No CPC-4 was observed in our study at hospital discharge.
At admission, significant differences were observed between CPC5 vs. CPC1, 2, and 3, and between CPC1 vs. CPC2 ($P < 0.01$). At H24 and H48, significant differences were observed between CPC5 vs. CPC1, 2, and 3.

Please cite this article in press as: N. Deye, et al., Protein S100B as a reliable tool for early prognostication after cardiac arrest, Resuscitation (2020), https://doi.org/10.1016/j.resuscitation.2020.08.010

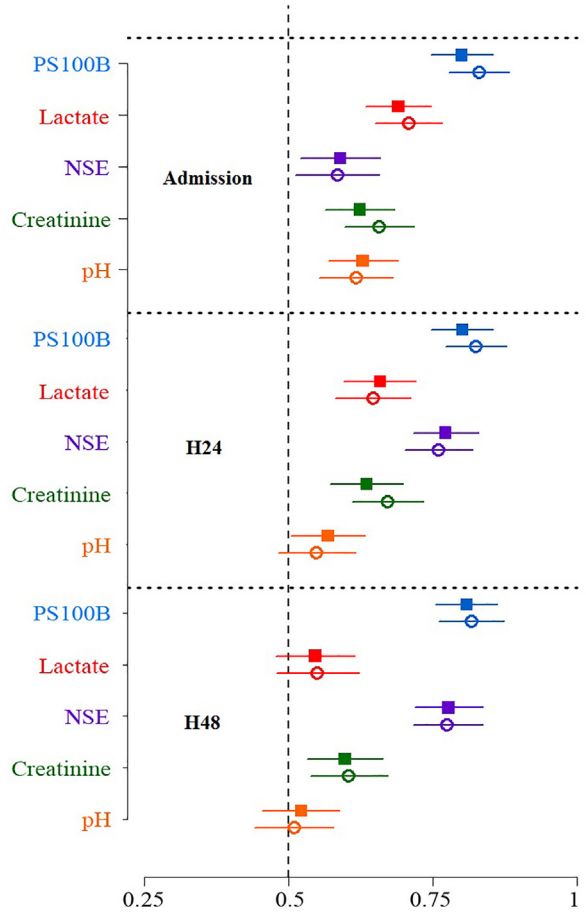

Fig. 2 – Forest plot for biomarker Receiver Operating Characteristic (ROC) curves from admission to H48 to predict outcome.
Biomarkers' Receiver Operating Characteristic (ROC) curves are represented with their area under the curve (AUC) and CI-95% at admission (upper part), H24 (middle part), and H48 (lower part) for their ability to discriminate good versus poor outcome at hospital discharge (black square) and at 3-month follow-up (white circle).
PS100B: protein S100B; NSE: Neuron specific Enolase.

guidelines using a multimodal approach performed after the first 2 days post-CA.

Some studies also suggested that PS100B could potentially help the early prognostication strategy,[29] confirming our findings that PS100B on admission was significantly associated with outcome. Combining NSE > 41.1 μg/L with PS100B > 0.461 μg/L at H24 was the most specific criteria of poor long-term outcome.[30] An initial PS100B threshold of 0.92 μg/L predicted poor outcome with a 90%-specificity, besides initial shockable rhythm, PS100B and NSE being highly predictive of hospital survival.[16] Mörtberg et al. observed that PS100B levels significantly differed between good and poor outcome groups as early as 1 h after ROSC, this difference persisting thereafter.[7] Only one study did not reach significance regarding the discriminative value for PS100B on admission, AUC of ROC curves on admission being lower than at H24 and H48.[31] However, its limited sample size could explain this observation. PS100B half-life is (0.5 to) 2 h with a molecular weight of 21 kDa, compared with 24 h and 78 kDa for NSE.[18,28] This possibly explains discrepancies in case of interrupted integrity of the blood-brain barrier after brain damages with an earlier release of PS100B into cerebral spinal fluid and blood.[32] More understanding of the "early" PS100B kinetic is warranted.

Serial PS100B measurements

In the first study published in 2001 before the TTM era and the modern post-CA syndrome management,[33] serial PS100B measurements showed significant differences over time according to outcome as soon as 30 min after resuscitation.. In the recent study by Duez et al.,[31] the daily change remained non-significant for PS100B, but the decrease was significant in the group showing good outcome. Other studies have previously described these PS100B variations in smaller cohorts.[7,19,34] Mörtberg suggested that levels of PS100B decreased in the good outcome group from the acute phase after ROSC to 24 h post-CA, and increased between these time points in the poor outcome group.[7] Einav et al. observed comparable results as ours with a "steep decrease" between admission compared to day 3, while the decrease between day 1 to day 3 was less pronounced.[19] Our study highlights the time course of PS100B within the first hours after CA and is to date the largest study confirming that serial PS100B

Table 3 – Time course of PS100B values between the first 3 days after CA according to outcome.

Δ PS100B	Good outcome: discharge	Poor outcome: discharge	Good outcome: M3	Poor outcome: M3	P[b] Discharge/M3
Δ Adm-H24 neg.	1[a]	23	1[a]	23	0.0001/0.001
Δ Adm-H24 pos.	62	84	51	84	
Δ Adm-H48 neg.	4	27	4	26	0.002/0.006
Δ Adm-H48 pos.	65	89	56	87	
Δ H24-H48 neg.	27	42	23	41	0.75/0.74
Δ H24-H48 pos.	43	77	37	77	

Abbreviation: Adm: day of initial sampling after ROSC, i.e. after admission; H24 corresponds to the following 24 h after the day of initial sampling, and H48 the following 24 h after H24; M3 denotes the 3rd month of follow-up after CA; Discharge denotes hospital discharge.
ΔPS100B Adm-H24 is defined as the difference between the first blood sample on admission (n = 278) and the sample at H24 (n = 257), the ΔPS100B Adm-H48 is the difference between the first blood sample on admission (n = 278) and the sample at H48 (n = 239), and ΔPS100B H24-H48 is the difference between the blood sample at H24 and the sample at H48. Pos. means a positive difference between the first and second samples (i.e. decreasing PS100B value measured on the second sampling). Neg. means a negative difference between the first and second samples (i.e. increasing PS100B value measured on the second sampling).
[a] PS100B values for this patient were 0.102 at D0 and 0.123 at D1.
[b] Fisher exact tests are expressed.

Please cite this article in press as: N. Deye, et al., Protein S100B as a reliable tool for early prognostication after cardiac arrest, Resuscitation (2020), https://doi.org/10.1016/j.resuscitation.2020.08.010

Table 4 – Biomarkers to predict outcome at 3 months after CA: sensitivity, specificity, AUC, NRI versus clinical model, and NRI versus clinical model + NSE-Adm.

Biomarker	Se[a]	Sp[a]	Criterion	AUC[b]	NRI (%) *vs.* clinical model[c]	NRI (%) *vs.* clinic + NSE[d]
PS100B Admission	76.7	79.0	0.539	0.831 [0.779–0.883]	69.8 [48.1–91.6]	63.8 [41.6–86.1]
PS100B H24	88.8	63.6	0.321	0.825 [0.772–0.879]	101.2 [79.5–122.9]	66.1 [36.2–96.0]
PS100B H48	79.5	75.9	0.139	0.818 [0.761–0.874]	53.4 [31.8–75.0]	97.8 [71.6–124.0]
NSE Admission	71.4	45.9	31.92	0.585 [0.512–0.658]	−21.7 [−46.3–2.9][e]	Reference
NSE H24	98.7	44.3	49.28	0.761 [0.702–0.820]	77.1 [52.9–101.3]	67.4 [37.5–97.2]
NSE H48	92.3	59.6	38.30	0.776 [0.716–0.837]	99.8 [77.7–121.8]	89.7 [64.4–115.1]
Lactate Admission	77.8	57.7	7.00	0.709 [0.650–0.767]	64.4 [42.5–86.2]	52.2 [27.0–77.5]
Lactate H24	71.3	55.0	2.77	0.647 [0.582–0.712]	42.2 [19.9–64.4]	19.1 [−5.8–44.1][e]
Lactate H48	31.6	82.8	1.24	0.551 [0.480–0.622]	34.2 [10.5–57.8]	−2.9 [−28.1–22.2][e]
pH Admission	57.0	63.9	7.23	0.618 [0.554–0.682]	57.6 [35.5–79.8]	57.6 [35.5–79.8]
pH H24	82.7	29.7	7.23	0.549 [0.483–0.616]	22.8 [−0.8–46.4] [e]	−7.5 [−31.1–16.1][e]
pH H48	77.2	36.4	7.32	0.510 [0.442–0.578]	32.8 [9.4–56.2]	−6.0 [−30.9–18.9][e]
Creat. Admission	85.2	44.7	126	0.658 [0.598–0.718]	67.6 [48.0–87.2]	32.9 [8.4–57.4]
Creat. H24	70.2	62.8	81	0.672 [0.610–0.735]	51.7 [29.5–73.8]	32.3 [7.5–57.1]
Creat. H48	84.5	42.9	116	0.605 [0.539–0.672]	42.3 [21.6–63.0]	30.2 [8.0–52.4]

Abbreviation. Se: sensitivity. Sp: specificity. AUC: area under the curve. NRI: Net Reclassification Index.
[a] Best sensitivity/specificity (with their criterion) according to the maximum accuracy.
[b] Biomarkers Receiver Operating Characteristic (ROC) curves are represented with their area under the curve (AUC) and CI-95% for their ability to discriminate good versus poor outcome at 3-month follow-up.
[c] Net Reclassification Index (NRI) of each biomarker at all time-points against clinical model (including clinical parameters with statistical significance in the multivariate analysis: initial shockable rhythm, no-flow duration, clinical seizures, and therapeutic hypothermia).
[d] NRI against clinical model (with parameters with statistical significance in the multivariate analysis) + NSE-Adm as the reference biomarker.
[e] No added value according to NRI. Biomarkers with NRI > 50% with and without NSE are PS100B at all time-points, NSE after H24, and pH and lactate on admission.

measurements, especially in the early phase after CA, can correctly predict outcome.

Biomarkers and scores for early prognostication

Our study is the first to evaluate simultaneously all "usual" biomarkers using ROCs, these biomarkers being correlated with outcome but with lower AUCs than PS100B-Adm. Interestingly, PS100B, pH and lactate were correlated in multivariate analyses, suggesting that these biomarkers could finally find a place in scores used in the early phase post-CA.[13] Creatinine remained significantly but moderately different between good and poor outcome groups, whereas pH was no longer significant at H24 and H48, neither lactate at H48. Our results are concordant with literature for lactate variations over time[12] and suggest that lactate or pH could be useful predictors early after CA but less thereafter. Except PS100B-Adm, overlaps for these biomarkers between good and poor outcome were important as early as admission. In our study, only PS100B was blinded to physicians preventing the risk of "self-fulfilling prophecy". Therefore, according to literature and our data, early PS100B could interestingly be incorporated in such prognosticating scores.

Limitations

Our study is a single-center study. However, regarding our main endpoint, this fact guarantees a correct homogeneity of samplings and biochemical tests, considering that the ECLIA method with immuno-luminescent sandwich used in our unit is the most used method in literature.[8,19,22,28,31] First sampling was performed after a 4-h delay post-CA, consequence of the important deployment of resources performed to find the aetiology of CA.[2,3] Choice of the days of sampling could be criticized as we did not pursue the biomarker evaluation after H48. However, our main goal was to specifically evaluate biomarkers in the early phase after CA.

Several exclusion criteria were prospectively applied in our study. However, this selection of patients added precision, as biomarkers could be modified by ECLS and haemolysis.[4] Both OHCA and IHCA were enrolled in our pragmatic trial. However, similar results were observed in the OHCA subgroup (data not shown). It could be argued that extra-cerebral sources of PS100B are potentially confounders, decreasing its specificity to predict outcome.[32] However, in our study, neither body mass index nor CPK were associated with PS100B values at admission (data not shown).

Finally, the cost of PS100B dosages, the duration of its measurement, or its unavailability in some laboratories could limit its spreading.[15] Meanwhile, more studies are warranted before any generalization of PS100B measurement: multicentre prospective trials to definitively confirm our results, refining early PS100B added value and its cut-off levels, and meta-analyses to pool all PS100B data in its ability to early predict outcome.

Conclusions

In our cohort of comatose patients resuscitated from CA without ECLS implementation, PS100B after hospital admission was the biomarker with the best accuracy for outcome's prognostication, and the sole biomarker with a high accuracy persisting during the first 3 days after CA.

Funding

No specific grant was received for this study. However, this study was performed under the coordination of both "Inserm UMR S-942 et le

Centres des Ressources Biologiques" of Lariboisiere University Hospital, Paris, and the doctoral school "Frontieres du Vivant, Centre de Recherches Interdisciplinaires", Paris, France.

Conflict of interest statement and disclosures

All the authors declare no competing interest in relation with the paper.

Outside the present work, Nicolas Deye (corresponding author) declared past lecture and travel fees from Bard and ongoing lecture and travel fees from Zoll Company.

CRediT authorship contribution statement

Nicolas Deye: Conceptualization, Methodology, Supervision, Data curation, Formal analysis, Writing - original draft, Validation. **Philippe Nguyen:** Data curation, Formal analysis, Writing - original draft, Validation. **Nicolas Vodovar:** Conceptualization, Methodology, Supervision, Formal analysis, Writing - original draft, Validation. **Malha Sadoune:** Data curation, Validation. **Corinne Collet:** Data curation, Formal analysis, Writing - original draft, Validation. **Sebastian Voicu:** Data curation, Formal analysis, Writing - original draft, Validation. **Isabelle Malissin:** Data curation, Writing - original draft, Validation. **Etienne Gayat:** Formal analysis, Writing - original draft, Validation. **Jeanne-Lise Samuel:** Conceptualization, Validation. **Claude Delcayre:** Conceptualization, Validation. **Jean-Marie Launay:** Conceptualization, Validation. **Alain Cohen-Solal:** Conceptualization, Validation. **Bruno Mégarbane:** Writing - original draft, Validation. **Alexandre Mebazaa:** Conceptualization, Methodology, Supervision, Formal analysis, Writing - original draft, Validation.

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.2020.08.010.

REFERENCES

1. Bougouin W, Lamhaut L, Marijon E, et al. Characteristics and prognosis of sudden cardiac death in Greater Paris: population-based approach from the Paris Sudden Death Expertise Center (Paris-SDEC). Intensive Care Med 2014;40:846–54.
2. Monsieurs KG, Nolan JP, Bossaert LL, et al. European Resuscitation Council Guidelines for Resuscitation 2015 Section 1. Executive summary. Resuscitation 2015;95:951–80.
3. 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. Resuscitation 2015;95:202–22.
4. Sandroni C, Cavallaro F, Callaway CW, et al. Predictors of poor neurological outcome in adult comatose survivors of cardiac arrest: A systematic review and meta-analysis. Part 2: Patients treated with therapeutic hypothermia. Resuscitation 2013;84:1324–38.
5. Wijdicks EF, Hijdra A, Young GB, Bassetti CL, Wiebe S, Quality Standards Subcommittee of the American Academy of Neurology. Practice parameter: prediction of outcome in comatose survivors after cardiopulmonary resuscitation (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2006;67:203–10.
6. Wiberg S, Hassager C, Stammet P, et al. Single versus serial measurements of Neuron-Specific Enolase and prediction of poor neurological outcome in persistently unconscious patients after out-of-hospital cardiac arrest - A TTM-trial substudy. Plos One 2017;12:e0168894.
7. Mörtberg E, Zetterberg H, Nordmark J, Blennow K, Rosengren L, Rubertsson S. S-100B is superior to NSE, BDNF and GFAP in predicting outcome of resuscitation from cardiac arrest with hypothermia treatment. Resuscitation 2011;82:26–31.
8. 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. J Am Coll Cardiol 2015;65:2104–14.
9. Vondrakova D, Kruger A, Janotka M, et al. Association of neuron-specific enolase values with outcomes in cardiac arrest survivors is dependent on the time of sample collection. Crit Care 2017;21:172.
10. Hasper D, von Haehling S, Storm C, Jörres A, Schefold JC. Changes in serum creatinine in the first 24 hours after cardiac arrest indicate prognosis: an observational cohort study. Crit Care 2009;13:R168.
11. Shinozaki K, Odaa S, Sadahiroa T, et al. Blood ammonia and lactate levels on hospital arrival as a predictive biomarker in patients with out-of-hospital cardiac arrest. Resuscitation 2011;82:404–9.
12. Dell'Anna A, Sandroni C, Lamanna I, et al. Prognostic implications of blood lactate concentrations after cardiac arrest: a retrospective study. Ann Intensive Care 2017;7:101.
13. Maupain C, Bougouin W, Lamhaut L, et al. The CAHP (Cardiac Arrest Hospital Prognosis) score: a tool for risk stratification after out-of-hospital cardiac arrest. Eur Heart J 2016;37:3222–8.
14. Shinozaki K, Oda S, Sadahiro T, et al. S-100B and neuron-specific enolase as predictors of neurological outcome in patients after cardiac arrest and return of spontaneous circulation: a systematic review. Critical Care 2009;13:R121.
15. Deye N, Vincent F, Michel P, et al. Changes in cardiac arrest patients' temperature management after the 2013 "TTM" trial: results from an international survey. Ann Intensive Care 2016;6:4.
16. Derwall M, Stoppe C, Brücken D, Rossaint R, Fries M. Changes in S-100 protein serum levels in survivors of out-of-hospital cardiac arrest treated with mild therapeutic hypothermia: a prospective, observational study. Critical Care 2009;13:R58.
17. Shinozaki K, Oda S, Sadahiro T, et al. Serum S100B is superior to neuron-specific enolase as an early prognostic biomarker for neurological outcome following cardiopulmonary resuscitation. Resuscitation 2009;80:870–5.
18. Wojtczak-Soska K, Lelonek M. S-100B protein: An early prognostic marker after cardiac arrest. Cardiol J 2010;17:532–6.
19. Einav S, Kaufman N, Algur N, Kark JD. Modeling serum biomarkers S100 beta and neuron-specific enolase as predictors of outcome after out-of-hospital cardiac arrest. An aid to clinical decision making. J Am Coll Cardiol 2012;60:304–11.
20. Einav S, Kaufman N, Algur N, Strauss-Liviatan N, Kark JD. Brain biomarkers and management of uncertainty in predicting outcome of cardiopulmonary resuscitation: a nomogram paints a thousand words. Resuscitation 2013;84:1083–8.
21. Rundgren M, Karlsson T, Nielsen N, Cronberg T, Johnsson P, Friberg H. Neuron specific enolase and S-100B as predictors of outcome after cardiac arrest and induced hypothermia. Resuscitation 2009;80:784–9.
22. Stammet P, Dankiewicz J, Nielsen N, et al. Protein S100 as outcome predictor after out-of-hospital cardfiac arrest and targeted temperature management at 33°C and 36°C. Critical Care 2017;21:153.
23. Nielsen N, Wetterslev J, Cronberg T, et al. Targeted temperature management at 33°C versus 36°C after cardiac arrest. N Engl J Med 2013;369:2197–206.
24. Voicu S, Deye N, Malissin I, et al. Influence of α-stat and pH-stat blood gas management strategies on cerebral blood flow and oxygenation in patients treated with therapeutic hypothermia after out-of-hospital cardiac arrest: a crossover study. Crit Care Med 2014;42:1849–61.
25. 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.

26. Sandroni C, Cavallaro F, Callaway CW, et al. Predictors of poor neurological outcome in adult comatose survivors of cardiac arrest: A systematic review and meta-analysis. Part 1: patients not treated with therapeutic hypothermia. Resuscitation 2013;84:1310–23.
27. Tianen M, Roine RO, Pettila V, Takkunen O. Serum neuron-specific enolase and S-100B protein in cardiac arrest patients treated with therapeutic hypothermia. Stroke 2003;34:2881–6.
28. Stammet P, Wagner DR, Gilson G, Devaux Y. Modeling Serum level of S100B and Bispectral Index to predict outcome after cardiac arrest. J Am Coll Cardiol 2013;62:851–8.
29. Calderon LM, Guyette FX, Doshi AA, Callaway CW, Rittenberger JC. Combining NSE and S100B with clinical examination findings to predict survival after resuscitation from cardiac arrest. Resuscitation 2014;85:1025–9.
30. Zellner T, Gärtner R, Schopohl J, Angstwurm M. NSE and S-100B are not sufficiently predictive of neurologic outcome after therapeutic hypothermia for cardiac arrest. Resuscitation 2013;84:1382–6.
31. Duez CHV, Grejs AM, Jeppesen AN, et al. Neuron-specific enolase and S-100b in prolonged targeted temperature management after cardiac arrest: a randomised study. Resuscitation 2018;122:79–86.
32. Gul SS, Huesgen KW, Wang KK, Mark K, Tyndall JA. Prognostic utility of neuroinjury biomarkers in post out-of-hospital cardiac arrest (OHCA) patient management. Med Hypotheses 2017;105:34–47.
33. Böttiger BW, Möbes S, Glätzer R, et al. Astroglial protein S-100 is an early and sensitive marker of hypoxic brain damage and outcome after cardiac arrest in humans. Circulation 2001;103:2694–8.
34. Huang CA, Tsai MS, Chien KL, et al. Predicting the outcome for out-of-hospital patients using multiple biomarkers and suspension microarray assays. Sci Rep 2016;6:27187.

Please cite this article in press as: N. Deye, et al., Protein S100B as a reliable tool for early prognostication after cardiac arrest, Resuscitation (2020), https://doi.org/10.1016/j.resuscitation.2020.08.010