Heliyon 10 (2024) e34618

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Validation of neuron-specific enolase in cardiac arrest patients with limited withdrawal of life-sustaining therapy

Dong Hun Lee [a,b], Byung Kook Lee [a,b,*], Yong Soo Cho [a], Dong Ki Kim [a], Seok Jin Ryu [a], Jin Hong Min [c], Jung Soo Park [c], Kyung Woon Jeung [a,b]

[a] Department of Emergency Medicine, Chonnam National University Hospital, Gwangju, Republic of Korea
[b] Department of Emergency Medicine, Chonnam National University Medical School, Gwangju, Republic of Korea
[c] Department of Emergency Medicine, College of Medicine, Chungnam National University, Daejeon, Republic of Korea

A B S T R A C T

Aim: We validated the prognostic performance of neuron-specific enolase (NSE) according to the recommended values in cardiac arrest (CA) survivors.

Methods: We analyzed the data of adult CA survivors who underwent targeted temperature management between January 2014 and December 2020. We measured the NSE level 48 h and 72 h after CA. We performed receiver operating characteristics (ROC) and used the reference value (17 μg/L) and the guidelines-suggested value (60 μg/L) as thresholds. The primary outcome was 6-month neurological outcomes with Cerebral Performance Category (CPC), dichotomized into good (CPC 1 or 2) or poor (CPC 3–5).

Results: Of the 513 included patients, 346 (67.4 %) patients had poor neurological outcomes. The area under ROC (AUC) of NSE at 48 h was 0.887 (95 % confidence intervals [CIs], 0.851–0.909) with the Youden index of 35.6 μg/L. A false positive rate (FPR) of <2 % was observed (54.1 μg/L). The thresholds values (17, 60) had a sensitivity of 86.1% and 56.7 % and a specificity of 66.7%and 98.8 %, respectively. The AUC of NSE at 72 h was 0.892 (95 % CIs, 0.849–0.920) with the Youden index of 30.4 μg/L. The threshold values (17, 60) had a sensitivity of 86.0%and 59.4 % with a specificity of 72.2%and 98.3 %, respectively. An FPR of <2 % was observed (53.6 μg/L). Among the 156 patients and 113 patients with NSE at 48 h and at 72 h ≤ 17 μg/L, respectively, 109 and 83 patients had good neurological outcomes.

Conclusions: The cut-off value of NSE (60 μg/L) was acceptable to predict poor neurological outcomes with an FPR <2 % in cardiac arrest survivors, irrespective of at 48 or 72 h. NSE (17 μg/L) can function as mitigating factor to deter early WLST.

1. Introduction

A majority of cardiac arrest patients either die or suffer from permanent neurological morbidity even after achieving the return of spontaneous circulation (ROSC) [1]. Early withdrawal of life-sustaining therapy (WLST) contributes to the increased mortality of cardiac arrest survivors [2]. Therefore, the guidelines suggest multimodal prognostic tools to avoid WLST for those who can potentially recover from neurological injury [3–5]. Finding patients predicted to have good neurological outcomes is also vital to efficient use of limited medical resources. Because advanced critical care, including mechanical circulatory support, consumes heavy medical resources, subjects should be carefully selected to avoid unnecessary efforts.

Among prognostic tools, biomarkers are more solid indicators than neurological exams or electrophysiologic studies, considering the advantages of being less affected by interference factors. Researchers have investigated various biomarkers, including neuron-specific enolase (NSE), s100B, neurofilament light chain protein, glial fibrillary acidic protein, and total-tau [6]. However, NSE is

* Corresponding author. Department of Emergency Medicine, Chonnam National University Medical School, 160, Baekseo-ro, Dong-gu, Gwangju, Republic of Korea.
E-mail address: bbukkuk@hanmail.net (B.K. Lee).

https://doi.org/10.1016/j.heliyon.2024.e34618
Received 18 October 2023; Received in revised form 12 July 2024; Accepted 12 July 2024
Available online 14 July 2024

the only biomarker recommended for use in guidelines due to its commercial utility and widespread application [3–5], despite the disadvantage that it might differ depending on the analysis method or equipment [7–13]. Although the studies that evaluated the prognostic performance of NSE reported different cut-off values [7,10–13], the reference value of NSE is < 17 μg/L and recent guidelines suggest that NSE >60 μg/L at 48 h and/or 72 h is a prognostic marker of poor outcome [3]. Premature WLST for neurological reasons in cardiac arrest patients is still common [2,14]. However, the setting without WLST is ideal to assess the performance of prognosticators in cardiac arrest patients. While WLST has been legally sanctioned in Korea since February 2018, its implementation remains infrequent. Despite issued European Resuscitation Council (ERC) recommendations [3], studies validating guidelines under different NSE analysis conditions and restricted WLST protocols are scarce. To validate NSE cut-off values, we analyzed the prognostic performance of different NSE cut-off values using single-center data in a setting with limited WLST. After applying the optimal NSE value at 48 h, we elucidated whether there is any prognostic value of serial NSE measurements.

2. Methods

2.1. Study design and population

We conducted a retrospective analysis of comatose cardiac arrest survivors at Chonnam National University Hospital, a university-affiliated hospital in the Republic of Korea, between January 2014 and December 2020. The Institutional Review Board of Chonnam National University Hospital approved this study (CNUH-2022-316) with a waiver for informed consent.

We included adult (≥18 years) cardiac arrest survivors who underwent targeted temperature management (TTM) for whom neuron-specific enolase was measured. We excluded those patients who had a Cerebral Performance Category (CPC) scale ≥3 before cardiac arrest, had extracorporeal membrane oxygenation (ECMO), died within 48 h after cardiac arrest, had missing data on NSE both at 48 h and 72 h, or were performed WLST. Instances of patients regaining consciousness, making NSE assessment unnecessary, patients dying, preventing blood sample collection, or the inability to obtain blood samples were classified as missing data.

2.2. Withdrawal of life-sustaining therapy and neuron-specific enolase measurement

Withdrawal of life-sustaining therapy was only permitted for patients in brain dead conditions before February 2018. The WLST has been enabled in the Republic of Korea by law, for patients with no chance of recovery, no recovery from treatment, rapid deterioration, and imminent death since February 2018. Based on multimodal prognostication incorporating NSE, brain computed tomography, amplitude-integrated electroencephalography, and neurological examination, we elected to WLST as per the written directive of the patient and/or at the request of their next-of-kin.

Blood samples were drawn at 48 h and 72 h after ROSC to measure NSE levels. Evaluation for hemolysis and subsequent correction in blood samples for NSE analysis were not conducted. The NSE levels were analyzed in our hospital laboratory by an immunoradiometric assay (IRMA) using a Riakey NSE IRMA tube (Shin Jin Medics Inc., Goyang, Gyeonggi-do, Korea) with Gamma 10 (Shin Jin Medics Inc., Goyang, Gyeonggi-do, Korea).

2.3. Data collection and outcomes

We obtained the following data from electronic medical records: age, sex, pre-existing illness, a witness of collapse, bystander cardiopulmonary resuscitation (CPR), first monitored rhythm, etiology of cardiac arrest, epinephrine dose, time to ROSC, serum lactate and glucose level after ROSC, PaO_2 and $PaCO_2$ after ROSC, sequential organ failure assessment (SOFA) score at the first day of cardiac arrest [15], and the NSE levels 48 h and 72 h after ROSC.

We assessed neurologic outcomes using the CPC scale six months after cardiac arrest via phone interview or medical records for whom died in hospital and recorded them as CPC 1 (good performance), CPC 2 (moderate disability), CPC 3 (severe disability), CPC 4 (vegetative state), or CPC 5 (brain death or death) [16]. The primary outcome was poor neurological outcomes, as CPC 3–5.

2.4. Statistical analysis

We reported counts with percentiles for categorical variables and median with interquartile ranges for continuous variables. We used the Chi-square test with continuity correction for 2 × 2 tables or Fisher's Exact test for categorical variables and the Mann-Whitney *U* test for continuous variables. We performed the receiver operating characteristics (ROC) to assess the prognostic performance of NSE. The Youden index is the point where the difference between the true positive rate and the false positive rate is maximized. We utilized the reference value of NSE (<17 μg/L) and the cut-off values of NSE as suggested by the European Resuscitation Council (ERC) (<60 μg/L) [3] as a threshold and calculated the values for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), true negative, false negative, false positive, and true positive. The area under ROC (area under the curve; AUC), sensitivity, specificity, PPV, and NPV are presented with 95 % confidence intervals (CIs). We used the method of DeLong et al. to compare the dependent ROC curves. The difference between NSE at 48 h and at 72 h were calculated with the following formula; NSE at 48 h – NSE at 72 h. NSE clearance was calculated with the following formular; [(NSE at 48 h – NSE at 72 h)/NSE at 48 h]*100. Data were analyzed using IBM SPSS Statistics 27.0 for Windows (IBM Corp., Armonk, NY, USA) and MedCalc® (MedCalc Software Ltd. Ostend, Belgium). A two-sided significance level of 0.05 was used to indicate statistical significance.

3. Results

3.1. Study population and general characteristics

A total of 669 comatose cardiac arrest survivors underwent TTM after ROSC. Of those, five patients had a CPC 3 before cardiac arrest, 42 patients underwent ECMO, 86 patients died within 48 h after cardiac arrest, 14 patients had missing data on NSE both at 48 h and 72 h, and nine patients were performed WLST and were therefore excluded. Finally, we included 513 patients in the study (Fig. 1).

Table 1 illustrates baseline characteristics stratified by neurological outcomes. Poor neurological outcomes were observed in 346 (67.4 %) patients. The poor neurological outcome group was older (65.0 years [54.0–74.0] vs. 56.0 years [46.0–66.0]) with higher incidence of hypertension (50.0 % vs. 36.5 %), diabetes (35.5 % vs. 20.4 %), and renal impairment (13.9 % vs. 6.6 %). They had lower incidence of witness of collapse, initial shockable rhythm, and cardiac etiology (Table 1). The poor neurological outcome group had a longer time to ROSC, required more epinephrine, and exhibited higher level of serum lactate, glucose, $PaCO_2$ and SOFA (Table 1).

3.2. Prognostic performance of neuron-specific enolase at 48 h and 72 h

Neuron-specific enolase at 48 h was measured in 509 patients, with 344 (67.6 %) showing poor neurological outcomes (Fig. 1). The AUC of NSE at 48 h was 0.882 (95 % CIs, 0.851–0.909; Fig. 2A). The optimal cut-off determined by the Youden index was 35.6 μg/L, with a sensitivity of 71.5 % and specificity of 93.3 %. Table 2 displays the ROC analysis with 5 %, 2 %, and 0 % false-positive rate (FPR) for the cutoff values of NSE of 41.5 μg/L, 54.1 μg/L, and 67.5 μg/L, respectively. Table 3 provides ROC analyses with specific cut-off values of 17 μg/Land 60 μg/L. The NSE >60 μg/L had a higher specificity of 98.8 % (95 % CIs, 95.7–99.9). The NSE ≤17 μg/L was observed in 156 patients, with 109 (69.9 %) showing good neurological outcomes.

Neuron-specific enolase at 72 h was measured in 329 patients, with 214 (65.9 %) showing poor neurological outcomes (Fig. 1). The AUC of NSE at 72 h was 0.889 (95 % CIs, 0.849–0.920; Fig. 2B). The Youden index indicated that the optimal cut-off was 30.4 μg/L, with a sensitivity of 75.7 % and specificity of 90.4 %. Table 2 demonstrates ROC analysis with 5 %, 2 %, and 0 % FPR, with cutoff values of NSE of 46.6 μg/L, 53.6 μg/L, and 148.2 μg/L, respectively. Table 3 provides ROC analyses with specific cut-off values of 17 μg/Land 60 μg/L. The NSE >60 μg/L had a higher specificity of 98.3 (95 % CIs, 93.9–99.8). The NSE ≤17 μg/L was observed in 113 patients, with 83 (73.5 %) showing good neurological outcomes.

3.3. Changes in NSE values between 48 h and 72 h

Both NSE values at 48 h and 72 h were obtained in 325 patients, including 113 with good and 212 with poor neurological outcomes. The AUCs of NSE were 0.877 (95 % CIs, 0.836–0.910) at 48 h and 0.887 (95 % CIs, 0.847–0.919) at 72 h. The prognostic performance of NSE values at 48 h and 72 h were not different ($p = 0.441$).

Table 4 shows the changes in NSE values between 48 h and 72 h. The difference in NSE values between these time points was not

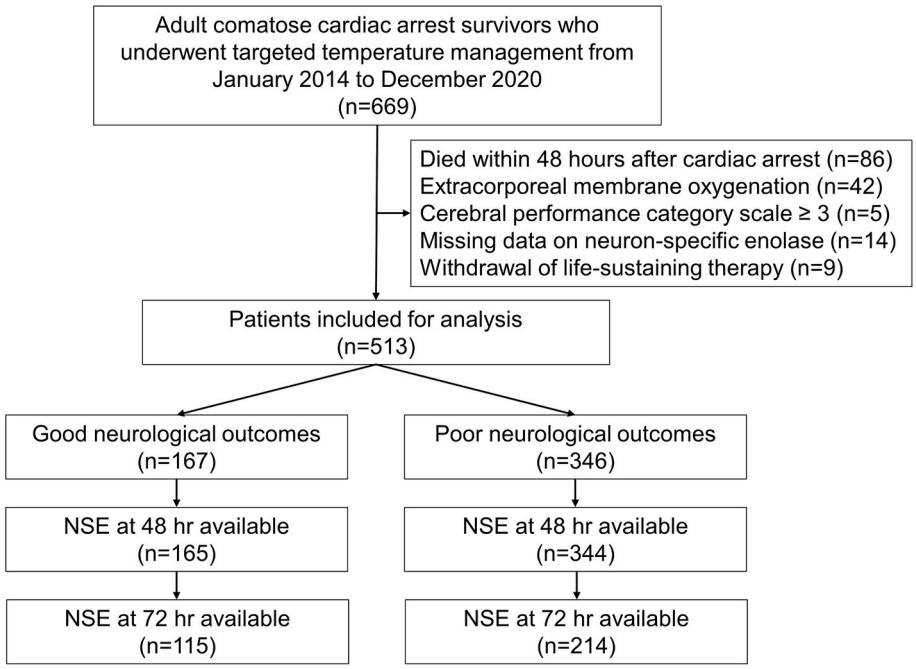

Fig. 1. Patient inclusion for analysis. NSE, neuron-specific enolase.

Table 1
Baseline characteristics stratified by neurological outcomes.

	Total (n = 513)	Good (n = 167)	Poor (n = 346)	*p*-value
Age, years	62.0 (51.0–72.0)	56.0 (46.0–66.0)	65.0 (54.0–74.0)	<0.001
Male, n (%)	344 (67.1)	121 (72.5)	223 (64.5)	0.088
Pre-existing illness, n (%)				
Coronary artery disease	91 (17.7)	32 (19.2)	59 (17.1)	0.644
Heart failure	38 (7.4)	7 (4.2)	31 (9.0)	0.080
Hypertension	234 (45.6)	61 (36.5)	173 (50.0)	0.005
Diabetes	157 (30.6)	34 (20.4)	123 (35.5)	0.001
Pulmonary disease	34 (6.6)	7 (4.2)	27 (7.8)	0.177
Renal impairment	59 (11.5)	11 (6.6)	48 (13.9)	0.023
Cerebrovascular accident	45 (8.8)	9 (5.4)	36 (10.4)	0.086
Liver cirrhosis	8 (1.5)	1 (0.6)	7 (2.0)	0.401
Cardiac arrest characteristics				
Witness of collapse, n (%)	369 (71.9)	138 (82.6)	231 (66.8)	<0.001
Bystander CPR, n (%)	376 (73.3)	125 (74.9)	251 (72.5)	0.655
Shockable rhythm, n (%)	160 (31.2)	108 (64.7)	52 (15.0)	<0.001
Cardiac etiology, n (%)	280 (54.6)	136 (81.4)	144 (41.6)	<0.001
Epinephrine, mg	2 (1–4)	1 (0–2)	3 (1–5)	<0.001
Time to ROSC, min	25.0 (15.0–39.0)	18.0 (11.0–28.0)	30.0 (17.0–44.0)	<0.001
Clinical characteristics after ROSC				
Lactate, mmol/L	7.8 (4.7–11.4)	6.5 (3.8–8.8)	8.4 (5.5–12.1)	<0.001
Glucose, mg/dL	235 (180–304)	216 (163–290)	243 (186–311)	0.021
PaO_2, mmHg	142 (83–247)	133 (80–220)	147 (86–256)	0.064
$PaCO_2$, mmHg	42.0 (33.0–52.0)	39.0 (34.0–46.0)	44.0 (33.0–56.0)	0.008
SOFA score	10 (7–12)	8 (6–10)	11 (8–12)	<0.001

CPR, cardiopulmonary resuscitation; ROSC, return of spontaneous circulation; SOFA, sequential organ failure assessment.

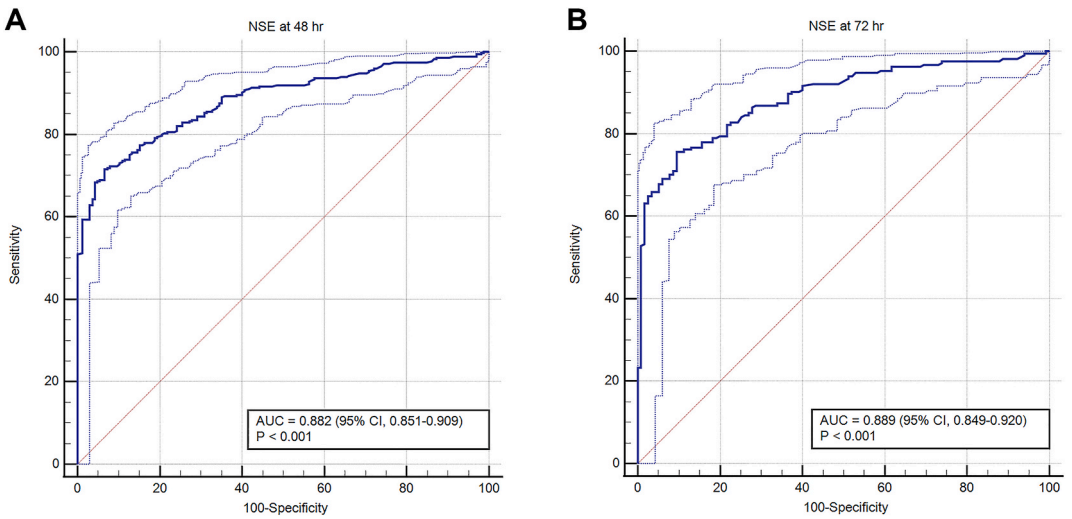

Fig. 2. Receiver operating characteristics (ROC) of neuron specific enolase values (NSE). The NSE at 48 h had an area under ROC (AUC) of 0.882 (95 % confidence intervals [CI]; 0.851–0.909) for 6-month poor neurological outcomes (A). The NSE at 72 h had an AUC of 0.889 (95 % CI; 0.849–0.920) for 6-month poor neurological outcomes (B).

Table 2
Receiver operating characteristics with 5 %, 2 %, and 0 % false positive rates.

NSE	FPR	Actual FPR (95 % CIs)	Cutoff	Sensitivity (95 % CIs)	PPV (95 % CIs)	NPV (95 % CIs)
48 h	≤5	4.8 (2.1–9.3)	41.5	68.6 (63.4–74.5)	96.7 (93.7–98.3)	59.2 (55.3–62.9)
	≤2	1.8 (0.4–5.2)	54.1	59.3 (53.9–64.5)	98.6 (95.7–99.5)	53.6 (50.4–56.8)
	0	0 (0–2.2)	67.5	50.9 (45.5–56.3)	100	49.4 (46.7–52.1)
72 h	≤5	4.3 (1.4–9.9)	46.6	65.9 (59.1–72.2)	96.6 (92.2–98.5)	60.1 (55.5–64.6)
	≤2	1.7 (0.2–6.1)	53.6	63.1 (56.2–69.6)	98.5 (94.5–99.6)	58.9 (54.5–63.1)
	0	0 (0–3.2)	148.2	23.4 (17.9–29.6)	100	41.2 (39.4–43.0)

NSE, neuron specific enolase; FPR, false positive rate; CIs, confidence intervals; PPV, positive predictive value; NPV, negative predictive value.

Table 3
Receiver operating characteristics with specific cutoff values of neuron-specific enolase at 48 h and 72 h for poor neurological outcomes.

NSE 48 h								
Values	Sensitivity (95 % CIs)	Specificity (95 % CIs)	PPV (95 % CIs)	NPV (95 % CIs)	TN	FN	FP	TP
>17	86.1 (81.9–89.5)	66.7 (58.9–73.8)	84.3 (81.2–87.0)	69.6 (63.3–75.3)	109	47	56	297
>60	56.7 (51.3–62.0)	98.8 (95.7–99.9)	99.0 (96.1–99.7)	52.2 (49.2–55.3)	163	148	2	196
NSE 72 h								
>17	86.0 (80.6–90.3)	72.2 (63.0–80.1)	85.2 (81.0–88.6)	73.5 (66.1–79.7)	83	30	32	184
>60	59.4 (52.4–66.0)	98.3 (93.9–99.8)	98.4 (94.1–99.6)	56.5 (52.4–60.5)	113	87	2	127

NSE, neuron specific enolase; CIs, confidence intervals; PPV, positive predictive value; NPV, negative predictive value; TN, true negative; FN, false negative; FP, false positive; TP, true positive.

different between neurological outcome groups (1.6 μg/L [−1.0 to 5.2] vs. 0.1 μg/L [−21.9 to 16.5]). However, more patients in the poor neurological outcome group had an increase in NSE levels (50.0 % vs. 31.9 %) and lower NSE clearance (0.6 % [−33.2 to 24.4] vs. 11.4 % [−9.6 to 29.9]) compared to the good neurological outcome group.

3.4. Withdrawal of life-sustaining therapy and organ donation

Withdrawal of life-sustaining therapy was performed in nine patients and organ donation was executed in five patients (Table 5). WLST within seven days after cardiac arrest was achieved in three patients and organ donation within seven days was completed in two patients.

4. Discussion

Neuron-specific enolase at 48 h and 72 h after ROSC had AUCs of 0.882 (95 % CIs, 0.851–0.909) and 0.889 (95 % CIs, 0.849–0.920) for predicting poor neurological outcome, respectively. The NSE at 48 h and 72 h after ROSC with cut-off values of <54.1 μg/L and <53.6 μg/L had a FPR less than 2 %. The difference in NSE value was not associated with neurological outcomes. The increase in NSE values between 48 h and 72 h and lower NSE clearance were associated with poor neurological outcomes. The 60 μg/L of NSE value that ERC recommended showed 98.8 % and 98.3 % of specificity to predict poor neurological outcomes in both NSE at 48 h and 72 h, respectively.

Neuron-specific enolase is a glycolytic enzyme abundant in gray matter neurons. Severe blood-brain barrier disruption with poor neurological outcomes following cardiac arrest was associated with elevated NSE values at 53–57 h after ROSC [17]. A systematic review also analyzed that NSE levels increase and peak 48–72 h after cardiac arrest [18]. A targeted temperature management trial reported the AUCs of NSE at 48 h and 72 h for 6-month neurological outcomes as 0.85 and 0.86, respectively [11]. An observational study that included 403 cardiac arrest patients revealed the AUCs of NSE at day two and day three for 2-year neurological outcomes were 0.81 and 0.85, respectively [8]. Another observational study that comprised 368 cardiac arrest patients displayed AUCs of NSE at 48 h and 72 h for 2–6 month neurological outcomes as 0.90 and 0.88, respectively [7]. A systematic review of the literature summarized that the AUCs of NSE at 24–48 h and at 48–72 h were 0.818 and 0.827 [10]. A meta-analysis including 9880 cardiac arrest survivors summarized that the AUC of NSE at 48 h was 0.84 [19]. Here, we reported the AUCs of NSE at 48 h and 72 h for 6-month neurological outcomes were 0.882 and 0.889, respectively. Although the AUC values of NSE can be different depending on outcome measurement timing, the prognostic performance seems to be similar within a range of very good performance.

The targeted temperature management trial described NSE ≥48 μg/L at 48 h and ≥38 μg/L at 72 h had an FPR <2 % for predicting poor neurological outcome [11]. Other studies that validated NSE values suggested higher NSE cut-off values to meet an FPR ≤2 % as 80–107.1 μg/L [7,12]. The NSE values with an FPR <2 % in the present study were 54.1 μg/L and 53.6 μg/L, which were closer to TTM trial results. The difference of NSE cut-off values among the studies might be based on the study design, clinical application of NSE for WLST, and outcomes. The treating physicians were blinded to NSE values in the TTM trial [20]. The TTM trial had 53 % poor neurological outcomes [20]. Although NSE alone was not used for the decision of WLST, NSE values were known to the treating physician and was used as one of the multimodal prognosticators in both observational studies [7,12]. The study by Streitberger et al. defined the primary outcome as CPC at intensive care unit (ICU) discharge, therefore, CPC 4 or 5 was defined as a poor neurological outcome, considering the possibility of recovery for the patients with CPC 3 [12]. Another study defined the primary outcome as CPC at 2–6 months after cardiac arrest and informed the poor neurological outcome as 68 % (249/368) [7]. We also stated 67 % for poor neurological outcome in the present study. However, we measured CPC at six months after cardiac arrest, which seems to be more

Table 4
Serial change of neuron-specific enolase between 48 h and 72 h according to neurological outcomes.

NSE values	Good (n = 113)	Poor (n = 212)	*p*-value
Δ between NSE 48 h and NSE 72 h, μg/L	1.6 (95 % CIs, −1.0 to 5.2)	0.1 (95 % CIs, −21.9 to 16.5)	0.191
Increase in NSE value	36 (31.9)	106 (50.0)	0.003
NSE clearance	11.4 (95 % CIs, −9.6 to 29.9)	0.6 (95 % CIs, −33.2 to 24.4)	0.004

NSE, neuron specific enolase; CIs, confidence intervals.

Table 5
Withdrawal of life-sustaining therapy and organ donation after cardiac arrest.

WLST	Age	Sex	NSE at 48 h (μg/L)	NSE at 72 h (μg/L)	Survival days after cardiac arrest
1	55	Male	5000	3000	12
2	52	Male	390	700	7
3	45	Male	63.6	89	13
4	78	Male	415	270	6
5	58	Female	172.1	86.9	44
6	47	Female	62	75	6
7	79	Male	415	550	17
8	80	Male	705		4
9	55	Male	63.3	71.1	15
Organ donation					
1	69	Male	211.6	600	8
2	56	Male	350	400	5
3	23	Male	760	570	5
4	35	Female	156	82.9	24
5	37	Female	198.2	116.6	8

WLST, withdrawal of life-sustaining therapy; NSE, neuron specific enolase.

consistent than CPC at ICU discharge or at 2–6 months after cardiac arrest for clinical outcomes in post-cardiac arrest patients. This might affect the validation of ERC recommendations of >60 μg/L. A meta-analysis also summarized FPR of 3 % with the cut-ff of 64.1 μg/L [19]. Both previous observational studies described FPRs of 4.3 % or ≥5 % with the cut-off of >60 μg/L [7,12]. However, the cut-off of >60 μg/L to meet an FPR <2 % was satisfactory at both the 48 h and 72 h NSE measurements in the present study. According to the ERC recommendations, our data indicated that NSE >60 μg/L might serve as a determinant for WLST for preserving medical resources. Conversely, for NSE <60 μg/L, delaying premature WLST and continuing the current treatment regimen are recommended to ensure the protection of patients with the potential for neurologic recovery.

Targeted temperature management trial evaluated whether the normal value of biomarker can be useful to distinguish the cardiac arrest survivors with good neurological outcomes and illustrated that NSE with normal value (≤17 μg/L) predicted good neurological outcome in 78.8 % [21]. Another observational study found that the 92 % of patients with normal value of NSE had CPC 1 to 3 at ICU discharge [12]. In the present study, we observed that 70 % (109/156) of patients and 73 % (83/113) of patients with normal NSE values at 48 and 72 h, respectively, exhibited good neurological outcomes. The normal level of NSE implies that it could function as a mitigating factor to deter premature WLST.

European Resuscitation Council recommendations also provided cumulative NSE values between time intervals as an adjunctive prognosticator [3]. However, the TTM trial failed to demonstrate that the combination of NSE at 48 h and NSE enhancement between 48 h and 72 h improved the prognostic performance as compared to NSE at 48 h alone [22]. The difference in NSE between 48 h and 72 h was not associated with neurological outcomes. However, the increase in NSE between 48 h and 72 h and lower NSE clearance were associated with poor neurological outcome in the present study. The sequential assessment of NSE has the capacity to mitigate false positives stemming from sample hemolysis. Compared to change in NSE between 24 and 48 h, the range of change between 48 and 72 h is not large [19,22]. In the patient with poor neurological outcomes, NSE increased more, and in the patients with good neurological outcomes, NSE remained low [19], so the change over time does not seem to suggest a high prognostic power as much as the NSE value.

Withdrawal of life-sustaining therapy contributes to mortalities in post-cardiac arrest patients and avoiding early WLST <72 h after cardiac arrest might save 16 % more neurological recovery [2]. An observational study that investigated awakening time of comatose cardiac arrest patients stated that about 18 % (88/477) of the awakening patients regained consciousness seven days after cardiac arrest [23]. Another prospective observational study revealed that the median awakening time of patients with good neurological outcome was six days [24]. Therefore, prognostic performance of NSE may vary depending on the timing of WLST. Among 460 deceased patients, the TTM trial administered WLST to 247 patients within seven days after cardiac arrest [20]. A study that validated NSE demonstrated that WLST was performed in 203 patients out of 234 deceased patients with a median survival time of four days (interquartile range; 3–7 days) [7]. Although a standardized protocol with multimodal prognostication was utilized to decide the WLST, a high proportion of patients died within seven days after cardiac arrest following a decision of WLST. Herein, only 14 out of 355 patients with poor neurological outcomes underwent WLST or organ donation. Excluding the five patients who were declared brain dead and donated their organs, WLST within seven days after cardiac arrest was only performed in four patients in the present study. The lower proportion of WLST in our study might strengthen the validity of the prognostic performance of NSE.

4.1. Strengths and limitations

This study had several strengths. It was conducted at a single institution, with NSE analysis performed in a single laboratory. Therefore, it ensures a high reliability of NSE values without bias from NSE analysis methods that might occur in multi-institutional studies. NSE analysis reagents and equipment from a different manufacturer than those used in previous studies created research conditions highly relevant to validating ERC recommendations. Although it was a single-institution study, the large sample size contributed to high statistical power. This study established the long-term neurological outcomes at 6 months as the primary outcome, including patients who did not undergo WLST, thus eliminating bias associated with early WLST. Additionally, patients who

underwent WLST were excluded from the analysis. However, the relatively low number of patients undergoing WLST resulted in a very low selection bias compared to the total number of patients.

This study had also several limitations. One limitation is that was a retrospective observational study performed in a single center. We can only verify the validity of NSE in our hospital with the ERC recommendations. Accounting for variations in NSE analysis methods and laboratory practices is essential. Even when analyzing NSE using the same reagents and equipment, the presence of differences between laboratories is uncertain. Moreover, substantial inter-laboratory discrepancies can be expected if different reagents and equipment are used. Further efforts should be made to validate the proposed guidelines across multiple institutions and countries to assess their general applicability. In addition, the potential measurement error of NSE due to hemolysis was not adjusted for in the analysis. The manufacturer of the IRMA is different from the previous studies, this might yield different results. Compared to NSE at 48 h, NSE at 72 h was missing in 180 (35 %) patients. Therefore, the validity of NSE at 72 h might be lower than that of NSE at 48 h. However, among patients with good neurological outcomes, all 52 patients missing NSE at 72 h had NSE $<$60 μg/L at 48 h. Given the 11.4 % NSE clearance rate in this group, their NSE at 72 h could have remained below 60 μg/L if measured. Conversely, 66 (50 %) out of 132 patients missing NSE at 72 h had NSE $>$60 μg/L at 48 h in the poor outcome group. Considering the 0.6 % clearance rate in this group, their NSE at 72 h could have been similar to their 48 h values if measured. Therefore, prognostic predictions could be valid for approximately 118 out of 184 patients (64 %) missing NSE at 72 h, suggesting the potential for improved prognostic performance of NSE at 72 h. The treating physician was not blinded to NSE measurements, which may have introduced bias through a self-fulfilling prophecy effect. However, the application of WLST was limited to only nine patients, and in five of them, WLST was performed more than seven days following cardiac arrest.

5. Conclusions

The cut-off value of 60 μg/L that ERC recommended is acceptable to predict poor neurological outcomes with an FPR $<$2 % in cardiac arrest patients who underwent TTM, irrespective of NSE at 48 h or at 72 h. Considering to use WLST as a decision-making tool for implementation or deferral is reasonable. A normal level NSE $\leq$17 μg/L can serve as a mitigating factor to deter early WLST.

Source of funding

This study was supported by a grant (BCRI23040) of Chonnam National University Hospital Biomedical Research Institute.

Data availability statement

The authors do not have permission to share data.

CRediT authorship contribution statement

Dong Hun Lee: Writing – original draft, Formal analysis, Data curation. **Byung Kook Lee:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition. **Yong Soo Cho:** Formal analysis, Data curation. **Dong Ki Kim:** Resources, Formal analysis, Data curation. **Seok Jin Ryu:** Resources, Formal analysis, Data curation. **Jin Hong Min:** Methodology, Investigation. **Jung Soo Park:** Writing – review & editing. **Kyung Woon Jeung:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Byung Kook Lee reports financial support was provided by Chonnam National University Hospital Biomedical Research Institute. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

None.

References

[1] C.W. Tsao, A.W. Aday, Z.I. Almarzooq, et al., Heart disease and stroke statistics-2022 update: a report from the American heart association, Circulation 145 (2022) e153–e639.
[2] J. Elmer, C. Torres, T.P. Aufderheide, et al., Association of early withdrawal of life-sustaining therapy for perceived neurological prognosis with mortality after cardiac arrest, Resuscitation 102 (2016) 127–135.
[3] J.P. Nolan, C. Sandroni, B.W. Bottiger, et al., European resuscitation Council and European society of intensive care medicine guidelines 2021: post-resuscitation care, Intensive Care Med. 47 (2021) 369–421.
[4] A.R. Panchal, J.A. Bartos, J.G. Cabanas, et al., Part 3: adult basic and advanced life support: 2020 American heart association guidelines for cardiopulmonary resuscitation and emergency cardiovascular care, Circulation 142 (2020) S366–S468.
[5] Y.M. Kim, K.W. Jeung, W.Y. Kim, et al., Korean guidelines for cardiopulmonary resuscitation. Part 5. Post-Cardiac arrest care, Clin Exp Emerg Med 2021 (8) (2020) S41–S64.

[6] M. Moseby-Knappe, T. Cronberg, Blood biomarkers of brain injury after cardiac arrest- A dynamic field, Resuscitation 156 (2020) 273–276.
[7] E. Lissner Ostlund, H. Levin, N. Nielsen, A. Frigyesi, A. Lybeck, Neuron-specific enolase and long-term neurological outcome after OHCA - a validation study, Resuscitation 168 (2021) 206–213.
[8] J. Muller, B. Bissmann, C. Becker, et al., Neuron-specific enolase (NSE) predicts long-term mortality in adult patients after cardiac arrest: results from a prospective trial, Medicines (Basel) 8 (2021) 72.
[9] S.M. Ryoo, Y.J. Kim, C.H. Sohn, S. Ahn, D.W. Seo, W.Y. Kim, Prognostic abilities of serial neuron-specific enolase and lactate and their combination in cardiac arrest survivors during targeted temperature management, J. Clin. Med. 9 (2020) 159.
[10] K. Sharma, M. John, S. Zhang, G. Gronseth, Serum neuron-specific enolase thresholds for predicting postcardiac arrest outcome: a systematic review and meta-analysis, Neurology 98 (2022) e62–e72.
[11] P. Stammet, O. Collignon, C. Hassager, 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. 65 (2015) 2104–2114.
[12] K.J. Streitberger, C. Leithner, M. Wattenberg, et al., Neuron-specific enolase predicts poor outcome after cardiac arrest and targeted temperature management: a multicenter study on 1,053 patients, Crit. Care Med. 45 (2017) 1145–1151.
[13] D. Vondrakova, A. Kruger, M. Janotka, et al., Association of neuron-specific enolase values with outcomes in cardiac arrest survivors is dependent on the time of sample collection, Crit. Care 21 (2017) 172.
[14] T.L. May, R. Ruthazer, R.R. Riker, et al., Early withdrawal of life support after resuscitation from cardiac arrest is common and may result in additional deaths, Resuscitation 139 (2019) 308–313.
[15] A.E. Jones, S. Trzeciak, J.A. Kline, The Sequential Organ Failure Assessment score for predicting outcome in patients with severe sepsis and evidence of hypoperfusion at the time of emergency department presentation, Crit. Care Med. 37 (2009) 1649–1654.
[16] C.M. Booth, R.H. Boone, G. Tomlinson, A.S. Detsky, Is this patient dead, vegetative, or severely neurologically impaired? Assessing outcome for comatose survivors of cardiac arrest, JAMA 291 (2004) 870–879.
[17] C. Kang, Y. You, H.J. Ahn, et al., Blood-brain barrier disruption as a cause of various serum neuron-specific enolase cut-off values for neurological prognosis in cardiac arrest patients, Sci. Rep. 12 (2022) 2186.
[18] C. Sandroni, S. D'Arrigo, S. Cacciola, et al., Prediction of poor neurological outcome in comatose survivors of cardiac arrest: a systematic review, Intensive Care Med. 46 (2020) 1803–1851.
[19] R.L. Hoiland, K.J.K. Rikhraj, S. Thiara, C. Fordyce, et al., Neurologic prognostication after cardiac arrest using brain biomarkers: a systematic review and meta-analysis, JAMA Neurol. 79 (2022) 390–398.
[20] N. Nielsen, J. Wetterslev, T. Cronberg, et al., Targeted temperature management at 33 degrees C versus 36 degrees C after cardiac arrest, N. Engl. J. Med. 369 (2013) 2197–2206.
[21] M. Moseby-Knappe, N. Mattsson-Carlgren, P. Stammet, et al., Serum markers of brain injury can predict good neurological outcome after out-of-hospital cardiac arrest, Intensive Care Med. 47 (2021) 984–994.
[22] S. Wiberg, C. Hassager, P. Stammet, 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 12 (2017) e0168894.
[23] D.H. Lee, Y.S. Cho, B.K. Lee, et al., Late awakening is common in settings without withdrawal of life-sustaining therapy in out-of-hospital cardiac arrest survivors who undergo targeted temperature management, Crit. Care Med. 50 (2022) 235–244.
[24] E.R. Nakstad, H. Staer-Jensen, H. Wimmer, et al., Late awakening, prognostic factors and long-term outcome in out-of-hospital cardiac arrest - results of the prospective Norwegian Cardio-Respiratory Arrest Study (NORCAST), Resuscitation 149 (2020) 170–179.