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Clinical paper

Neuron-specific-enolase as a predictor of the neurologic outcome after cardiopulmonary resuscitation in patients on ECMO

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Abstract

Background: Neuron-specific-enolase (NSE) is frequently used to predict the neurologic outcome in persistently unconscious patients after cardiopulmonary resuscitation (CPR). However, its predictive value is unclear in the setting of veno-arterial extracorporeal membrane oxygenation therapy (ECMO). Aim of this project is to evaluate the predictive value of NSE in ECMO patients.

Methods: NSE was measured after 24, 48, and 72 h in post-CPR ECMO patients. Neurologic status was evaluated using the best Cerebral Performance Categories Score (CPC) during the hospital stay. Patients who deceased within the first 24 h and patients who were awake during the first 24 h were excluded. ROC curves were calculated to assess the discriminative ability of single NSE measurements. Trajectories of serial NSE values were investigated using latent class mixed models.

Results: The derivation cohort consisted of 65 patients, 30-day all-cause mortality was 47.7% and a poor neurological outcome with a CPC score of 4–5 was seen 30.7%. NSE measurement after 48 h showed the best discrimination for poor neurological outcome (AUC of 0.87 in the ROC curve; cut-off value of 70 μg/L). Specificity was highest if using serial NSE measurements at all three time points. These results could be validated in an external cohort of 64 patients.

Conclusion: In post-CPR patients on ECMO, NSE can be used to assess the neurologic outcome. Importantly, specificity was highest if using serial NSE measurements. Further research using prospective datasets is needed to verify these findings.

Keywords: NSE, ECMO, Resuscitation, Neurologic outcome

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Introduction

Early prognostication of neurologic outcome is important after cardiopulmonary resuscitation (CPR) to avoid futile therapy. Due to the complexity of the disease, neurologic assessment in this situation is based on multiple factors.[1] However, as clinical examination of patients after CPR is often hindered by sedatives and muscle relaxants, especially after application of targeted temperature management, biomarkers are of increasing importance.[2]

Current international guidelines on post-CPR therapy recommend the usage of neuron-specific enolase (NSE) as an integral part of neurologic assessment.[1] NSE is the dominant protein of the brain's white matter and is thus released following brain injury, e.g. after hypoxic events.[3] NSE strongly correlates with the neurologic outcome of patients after CPR.[4–6] Importantly, although previously a NSE cut-off value of 33 μg/l after 48 h was used to predict a poor neurologic outcome, current guidelines do not recommend any specific cut-off value.[7] However, NSE is not only an integral part of the brain's white matter, but also an active protein in thrombocytes and erythrocytes.[8] Consequently, previous studies have shown an increase of serum NSE levels in concordance with markers of haemolysis.[9]

During the past decade, veno-arterial extracorporeal membrane oxygenation therapy (ECMO) has been increasingly used for treatment of cardiogenic shock and as a valid rescue option for patients with refractory cardiac arrest.[10–12] This leads to an increasing interference of ECMO and NSE measurements. As ECMO is associated with higher levels of haemolysis, the reliability of NSE as a diagnostic tool in this setting is under discussion.[13]

The aim of this study was to evaluate the diagnostic reliability of NSE as a predictive marker for the neurologic outcome during ECMO therapy. Therefore, ECMO-specific NSE cut-off values were established and compared in a derivation-validation setting.

Material and methods

The present study is in compliance with the Declaration of Helsinki and was approved by the local ethics committee.

Study design

The present study is a retrospective analysis of consecutive patients admitted to two intensive care units (ICU) for ECMO treatment of cardiogenic shock or refractory cardiac arrest and prior CPR. Only patients with ECMO implantation within 6 h after CPR or ECMO implantation during active CPR and with available NSE values were selected. Patients who were awake within 24 h after the index event and patients who deceased within 24 h after the index event were excluded from the analysis. Patients were followed-up during the hospital stay. All patients received best medical care according to guidelines.[1] Decision on withdrawal of care was based on multiple factors including NSE values, with the neurological examination being the most important aspect. For this analysis, NSE cut-off values were evaluated in a derivation cohort from the first hospital. Thereafter, the findings were tested in an external validation cohort from the second hospital.

NSE measurement

NSE is measured 48 and 72 h after cardiac arrest in every patient treated at both centres. NSE was also measured after 24 h in the derivation cohort. In case of haemolysis, the blood drawings were discarded. Analyses of NSE were performed using a chemiluminescence assay (DiaSorin, Saluggia, Italy). NSE analysis was performed directly after the blood draw.

Follow up and primary endpoint

Follow-up was conducted by review of medical files. The primary endpoint was the best neurologic outcome during hospital stay. Per standard operating procedure protocol, every patient is being evaluated regarding his neurologic status every 8 h at both centres. Additionally, every patient is being evaluated by a neurologist during the ICU stay. These examinations are stored in the electronic medical record database. For the analysis, poor neurologic outcome was defined as CPC score of 4–5 equivalent to coma, brain death and death. Importantly, CPC score of 3 during the index hospital stay was not counted to be a poor outcome, as these patients do have a chance of recovery during neurologic rehabilitation.

Statistical analyses

Continuous variables were described as median (25th, 75th percentile); categorical variables as absolute numbers and percentages. The Wilcoxon rank-sum (for continuous variables) or Fisher's exact test (for categorical variables) test were used for between-group comparisons. For each of the first three days after initiation of ECMO, discriminative ability of the single NSE measurement in predicting poor neurologic outcome was assessed by a receiver operating characteristic (ROC) curve and the respective area under the curve (AUC) in the derivation cohort. Optimal cut-off values of NSE were derived from each ROC curve by identifying the point with the lowest Euclidean distance to the point P (0|1). For these cut-offs values, sensitivity and specificity values were calculated.

To assess discriminative ability of serial NSE measurements, two steps were performed: first, latent class mixed models were used to investigate trajectories of NSE in the first three days after initiation of ECMO.[14] With LCMM, unobserved groups within the sample that have distinct patterns of change in NSE over time are modelled. Individuals are then classified as belonging to one of the groups based on individual trajectories. The number of groups and the order of the polynomial functions (referred to as "mixture") to model the trajectories have to be supplied by the analyst. For each combination of number of groups (2, 3, 4, or 5) and mixture (polynomial functions of order 1, 2, 3, 4, or 5), an LCMM was calculated and its Bayesian Information Criterion (BIC) and the proportion of patients in each class were assessed. The LCMM with the highest BIC that had no class with less than 10% of the patients was selected. This way, LCMM were modelled for untransformed NSE values as well as for the natural logarithm of NSE values. Second, depending on the trajectories selected in the first step, a measure of change in NSE was derived, e.g. difference between NSE values on two different days, and its AUC and optimal cut-off value were determined as described above. Finally, each optimal cut-off value of the single NSE measurements was combined with the optimal cut-off value of the measure of change in NSE. Predictive values were assessed for each combination of cut-off values. Hereafter, the above mentioned findings were tested in the validation cohort. All computations were performed with R version 3.4.3.

Results

Derivation cohort

Sixty-five patients treated with an ECMO between January 2012 and June 2017 were included in the derivation cohort (Fig. 1). Median age was 50.0 (45.0, 60.0) years, 75.4% were male. In 58.5% the underlying disease was acute myocardial infarction. CPR was performed prior to initiation of ECMO therapy with a median duration of CPR of 30.0 (20.0, 63.8) min in all patients. In 49.2% ECMO was implanted under ongoing CPR. Median lactate before initiation of ECMO therapy was 8.1 (4.6, 14.0) mmol/l. Targeted temperature management (33 °C for 24 h, rewarming rate of 0.25 °C/h) was used in 70.8% (Table 1).

External validation cohort

Sixty-four patients treated with an ECMO between January 2014 and April 2018 were included in the validation cohort (Fig. 1). Median age was 57.5 (50.0, 70.0) years, 82.8% were male. In 67.2% the underlying disease was acute myocardial infarction. CPR was performed prior to initiation of ECMO with a median duration of 55.0 (35.0, 70.0) min. In all patients the ECMO was implanted under ongoing CPR. Median lactate before initiation of ECMO therapy was 11.8 (8.5, 16.0) mmol/l. Targeted temperature management was used in all cases (Table 1).

Clinical course

Median duration of ECMO support in the derivation cohort were 7.0 (5.0, 11.5) days and 30-day all-cause mortality rate was 47.7%. In the external validation cohort, median duration of ECMO support were 3.0 (2.0, 5.0) days and 30-day all-cause mortality rate was 53.1%.

NSE measurements and neurological outcome

Median NSE levels after 24 h, 48 h and 72 h in the validation cohort were 46.7 (34.0, 94.8) μg/l, 47.8 (38.0, 77.3) μg/l and 41.4 (29.3, 53.5) μg/l, respectively. After 24 and 48 h, NSE values were available for all patients whereas NSE values were only available for 59 patients after 72 h.

Of the 65 patients, 69.3% had a CPC score of 1–3 and 30.7% had a CPC score of 4–5. NSE values were significantly higher in the group with the poor neurologic outcome at every time point (100.2 (55.1, 127.8) vs. 41.6 (30.5, 59.1) μg/l after 24 h; 93.7 (70.3, 180.5) vs. 44.0 (30.3, 53.0) μg/l after 48 h; 73.0 (40.4, 126.7) vs. 37.5 (26.7, 45.0) μg/l after 72 h; $p < 0.01$ at all time points).

In the external validation cohort, median NSE levels after 48 h and 72 h were 63.0 (37.2, 121.3) and 58.0 (31.8, 107.7) μg/l, respectively. In this cohort, 48.4% of the patients had a CPC score of 1–3 and 51.6% had a CPC score of 4–5. Again, NSE values were significantly higher in the group with the poor neurologic outcome at every time point (113.0 (65.0, 181.7) vs. 40.0 (25.2, 54.6) μg/l after 48 h; 92.0 (59.0, 211.2) vs. 32.0 (23.8, 54.5) μg/l after 72 h; $p < 0.01$ at all time points). After 48 h, NSE values were available for all patients whereas NSE values were only available for 53 patients after 72 h.

Single NSE measurement to predict the neurologic outcome

In the derivation cohort, the AUC of the ROC curves for the NSE measurement after 24 h was 0.78 with an optimal NSE cut-off value of 68.1 μg/l to predict a CPC score of 4–5. After 48 h, the AUC was 0.87 with an optimal NSE cut-off value of 70 μg/l. After 72 h, the AUC was 0.80 with an optimal NSE cut-off value of 56 μg/l (Fig. 2). Test accuracy of these newly derived NSE cut-off values is shown in Table 2.

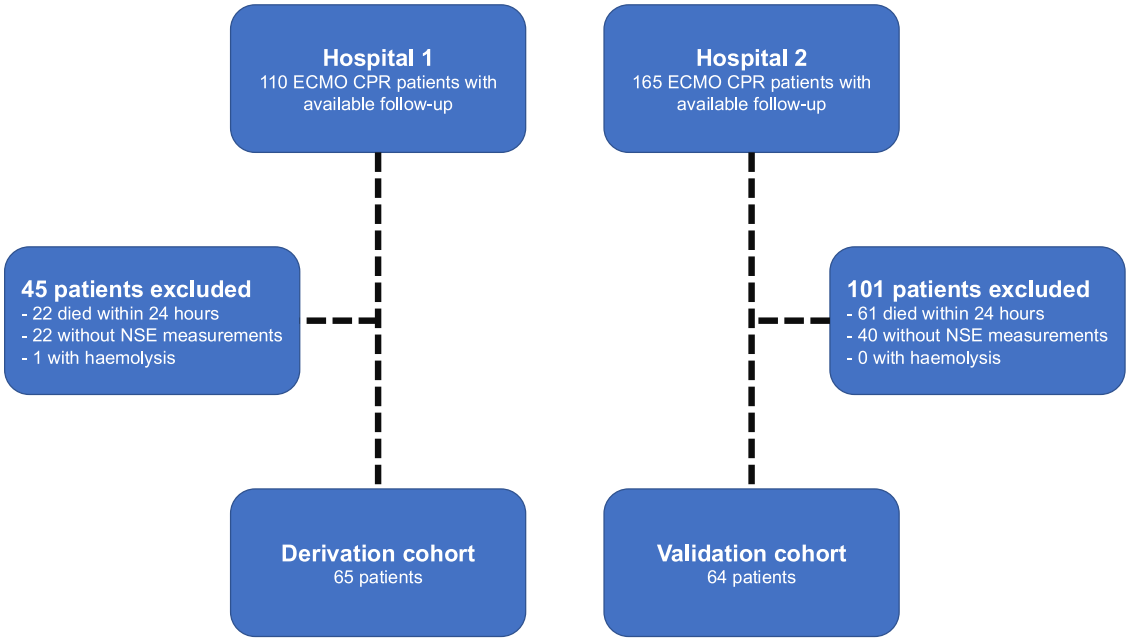

Fig. 1 – Flow chart of the study.

Table 1 – Baseline characteristics of the study populations.

	Derivation cohort (n = 65)	Validation cohort (n = 64)
Age (years)	50.0 (45.0, 60.0)	57.5 (50.0, 70.0)
Sex (male)	49 (75.4)	53 (82.8)
Cause of cardiogenic shock		
Acute myocardial infarction	38 (58.5)	43 (67.2)
Acute heart failure	17 (26.2)	10 (15.6)
Other	10 (15.3)	11 (17.2)
Cardiac arrest	65 (100)	64 (100)
Out of hospital	33 (50.8)	37 (57.8)
Total duration (min)	30.0 (20.0, 63.8)	55.0 (35.0, 70.0)
Refractory cardiac arrest	32 (49.2)	64 (100.0)
Lactate (mmol/l)	8.1 (4.6, 14.0)	11.8 (8.5, 16.0)
Creatinine clearance (ml/min)	51.3 (35.5, 65.2)	51.7 (42.2, 64.1)

Baseline characteristics of patients with cariogenic shock treated with ECMO therapy after cardiac arrest in both cohorts. Data are presented as median (25th, 75th percentile) or numbers (frequencies). ECMO = extracorporeal membrane oxygenation.

Table 2 – Test accuracy of the newly derived NSE cut-off values in the derivation cohort.

	Sensitivity (95% confidence interval)	Specificity (95% confidence interval)
24 h		
NSE > 68 µg/l	68.4 (43.4, 87.4)	78.9 (62.7, 90.4)
48 h		
NSE > 70 µg/l	76.5 (50.1, 93.2)	86.5 (71.2, 95.5)
72 h		
NSE > 56 µg/l	57.1 (28.9, 82.3)	94.1 (80.3, 99.3)

Test accuracy of the newly derives NSE cut-off values in patients on extracorporeal membrane oxygenation therapy after cardiac arrest in the derivation cohort. NSE = neuron-specific-enolase.

Serial NSE measurement to predict the neurologic outcome

In the derivation cohort, the latent class mixed models with two groups and third-order polynomial functions to model the logarithm of NSE showed the best model fit (BIC = 274.97, at least 23.1% of the sample in each group). Within the first group, serial NSE measurements showed a steady decline from day 1 to day 3. In contrast, the second group showed a trend-wise increase of NSE values from day 1 to day 3 (Fig. 3). Analysing the neurologic outcome of these groups, there was a strong trend towards a better neurologic outcome in group 1 (75.6 vs. 46.2%, p = 0.08).

Combination of single and serial NSE measurement

NSE at day 3 divided by NSE at day 2 was selected as the measure of change. The AUC of this measure was 0.56; a change of NSE > 92% from day 2 to day 3 was identified as optimal cut-off value to predict a CPC score of 4–5. The combination of each single NSE measurement with the optimal cut-off value of the measure of change in NSE further improved the specificity (Table 3). In clinical practice, this translates into NSE values which need to be a) above the respective cut-off value and b) need to be relatively stable or increasing from day 1 to day 2 to predict a CPC score of 4–5. 9.0%, 6.4%, and 9.0% of the patients were ruled-in after 24, 48, and 72 h, respectively, for a poor neurologic outcome.

Validation of single and serial NSE measurement

The NSE cut-off values evaluated in the derivation cohort were then tested using an external validation cohort. As in this cohort NSE values after 24 h were not present, the analysis was restricted to the time points of 48 h and 72 h. In the external validation, combining a single NSE measurement with the tested measure of change (NSE 72 h/NSE 48 h ≥ 92%) significantly improved the specificity (Table 4). These values were in accordance with those of the derivation cohort.

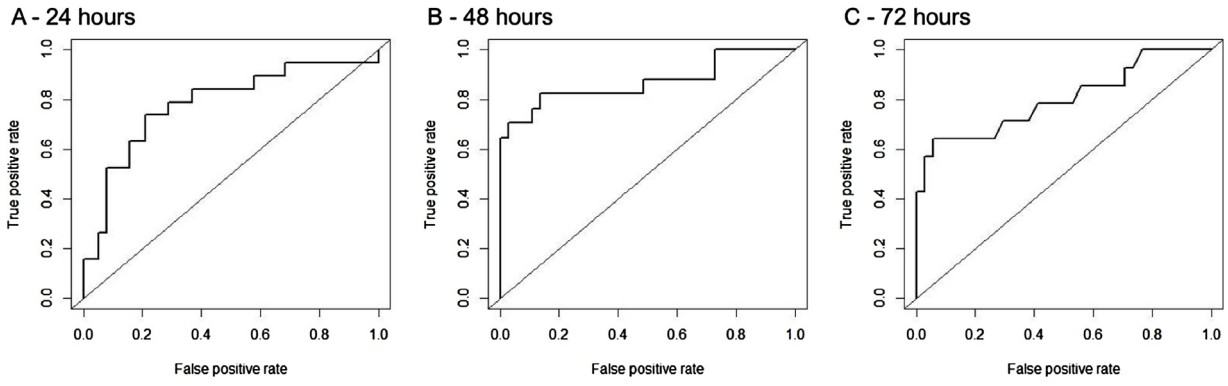

Fig. 2 – Receiver operator characteristic curves were used to investigate NSE as a predictive marker for the neurologic outcome, defined by the cerebral performance category score. The AUC for a NSE measurement after 24 h is 0.78 with an optimal NSE cut-off value of 68.1 µg/l to predict a CPC score of 1–3 (A). After 48 h, the AUC is 0.87 with an optimal NSE cut-off value of 70 µg/l (B). After 72 h, the AUC is 0.80 with an optimal NSE cut-off value of 56 µg/l (C). NSE = Neuron-Specific-Enolase; AUC = area under the curve; CPC = Clinical performance category.

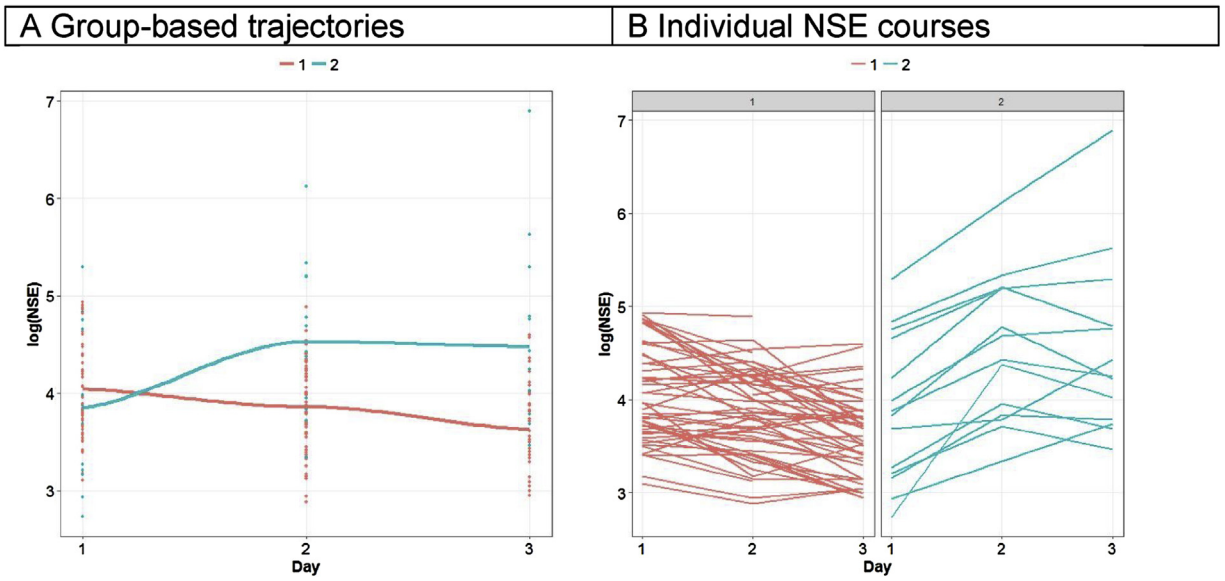

Fig. 3 – Two distinct groups could be defined using serial NSE measurements in the derivation cohort. The first group showed a steady decrease of NSE values from day 1 to day 3. In contrast, the second group showed a steady increase of NSE values. There was a strong trend towards a better neurologic outcome in group 1 as compared to group 2 (75.6 vs. 46.2%, p = 0.08). NSE = neuron-specific-enolase.

Discussion

The prime finding of this study is that NSE values can be used to evaluate the neurologic outcome in patients after CPR on ECMO as part of a multifactorial approach. Especially serial NSE measurements provided a high specificity in the presented analysis.

Early assessment of the neurologic outcome is crucial in the ICU to avoid futile treatment. This evaluation process is essential for decision making and should therefore be as reliable as possible. Evaluating the neurologic outcome is a multifactorial process. Biomarkers are an important part of this process and have two advantages: (a) They are not obscured by the use of sedatives/muscle relaxants. (b) They can be used early in the evaluation process.[7] However, the increase in ECMO therapy in these patients has led to insecurities regarding usage of NSE in this setting. As ECMO is inevitably linked to a certain degree of haemolysis, NSE values might be elevated as this protein can also be found in erythrocytes and thrombocytes.[13] Therefore, it is currently not known whether ECMO therapy just alters NSE values or renders the usage of this biomarkers completely pointless.

This study is the first to test distinct NSE cut-off values in patients after CPR on ECMO. In this analysis, we could show that single NSE measurements can be used to assess the neurologic outcome in patients after CPR on ECMO. The derived NSE cut-offs showed AUC values comparable to those derive from the TTM trial with a high specificity after 48 and 72 h. Notably, overall NSE values in our study were higher as in the TTM trial.[15] Importantly, using serial NSE measurements, we were able to depict two distinct trends within the study group. Hereby, patients with a steady decrease in NSE showed a strong trend towards a better neurologic outcome as

Table 3 – Combination of single and serial NSE measurements in the derivation cohort.

	Sensitivity (95% confidence interval)	Specificity (95% confidence interval)	% ruled-in (95% confidence interval)
24 h NSE > 68 µg/l + change > 92%	35.7 (12.8, 64.9)	94.7 (82.3, 99.4)	9.0 (3.7, 17.6)
48 h NSE > 70 µg/l + change > 92%	35.7 (12.8, 64.9)	100.0 (90.5, 100.0)	6.4 (2.1, 14.3)
72 h NSE > 56 µg/l + change > 92%	42.9 (17.7, 71.1)	97.1 (84.7, 99.9)	9.0 (3.7, 17.6)

Combination of the NSE cut-off values after 24, 48 and 72 h with the optimal cut-off value of the measure of change in NSE in the derivation cohort. A change of NSE ≥ 92% from day 2 to day 3 (i.e. NSE at day 3 divided by NSE at day 2) has been identified as optimal. Specificity at each time point is improved significantly. In clinical practice, this translates in NSE values which are (a) above the respective cut-off value and (b) are relatively stable or increasing from day 2 to day 3. NSE = neuron-specific-enolase.

Table 4 – External validation of single and serial NSE measurements.

	Sensitivity (95% confidence interval)	P	Specificity (95% confidence interval)	P
48 h				
NSE > 70	72.7 (54.5, 86.7)	1.00	77.4 (58.9, 90.4)	0.36
NSE > 70 µg/l + change > 92%	37.9 (20.7, 57.7)	1.00	93.5 (78.6, 99.2)	0.20
72 h				
NSE > 56 µg/l	77.8 (57.7, 91.4)	0.28	76.9 (56.4, 91.0)	0.07
NSE > 56 µg/l + change > 92%	44.4 (25.5, 64.7)	1.00	92.3 (74.9, 99.1)	0.57

External validation of the NSE cut-off values evaluated in the derivation cohort. Single NSE measurements as well as serial NSE measurements were tested. As in the derivational cohort, serial NSE measurement showed sufficient specificity. Additionally, Chi-squared testing with non-significant changes between both cohorts regarding test accuracy measures indicates a successful validation of the NSE cut-off values. NSE = neuron-specific-enolase.

compared to patients with steadily increasing NSE values. Combining these findings with each day's optimal cut-off value further enhanced the specificity. Together with a previous study linking ECMO therapy to higher NSE values,[13] the findings from the serial NSE measurements indicate that ECMO therapy produces a NSE "noise" which elevates the absolute values. However, this does not obscure the relevant release of NSE from the brain's white matter in the case of severe brain injury. Noteworthy, the accuracy of the above presented NSE cut-off values to predict a poor neurologic outcome could be validated in an external cohort. This further enhances the validity of our findings.

Recently, Floerchinger et al. reported on NSE in 131 patients after cardiac arrest and on ECMO. The neurologic outcome was not assessed clinically using the CPC score but by cerebral computer-tomography (CT) scan.[16] In this study, overall NSE values were significantly higher as expected from non-ECMO patient, too. Additionally, an increase of NSE values within 48 h as well as very high NSE values were associated with a higher chance of neuronal damage detected by the CT scan. These findings do support the above presented results, as the higher chance of neuronal damage does most likely explain the poor neurologic outcome.

In comparison to studies on NSE in non-ECMO patients, there are some similarities, too. First of all, highest NSE values in the overall cohort were observed after 48 h. This finding is consistent to the TTM trial, a prospective trial of targeted temperature management in 686 patients after cardiac arrest.[15] Secondly, a trend-wise increase was associated with a worse neurologic outcome in non-ECMO patients, too.[17] Thirdly, the AUC in the ROC curve analysis presented above are well in the range of those in non-ECMO patients.[15,17] These observations further validate the results from our analysis.

However, albeit the usage of a validation cohort provides a sophisticated testing of newly derived cut-off values, it is important to state that there are some limitations. First, the sample size of this study is relatively small as compared to the guideline-relevant studies on NSE usage on non ECMO patients. Secondly, previous studies on this topic mainly used "CPC score at discharge" as the primary outcome. Although "best CPC score during hospital stay" might provide a more accurate information on the impact of the initial event and was therefore chosen for this analysis, this might impact comparability. Additionally, non-blinded studies on diagnostic tools might always be biased. Therefore, our study can only be seen as explorative and the findings interpreted as hypothesis generating. A prospective, blinded trial is utterly needed to confirm the above presented results.

Importantly, NSE may not be used as a stand-alone tool to evaluate patients after CPR, as no specific NSE threshold for prediction of poor outcome with 0% false positive rate can be recommended at present. The decision to limit ICU therapy should always be based on a multifactorial process.

Conclusion

Despite ECMO treatment, NSE might still be a valuable tool to assess the neurologic outcome after CPR. Importantly, serial NSE measurements provided the highest specificity. These findings should be validated in a prospective, blinded trial to further investigate this important topic.

Conflict of interest

There are no conflict of interest to declare.

Sources of funding

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