Epilepsy & Behavior 101 (2019) 106432

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Serum neurofilament light chain as a prognostic marker in postanoxic encephalopathy

Giulio Disanto [a], Chiara Prosperetti [a], Claudio Gobbi [a], Christian Barro [b], Zuzanna Michalak [b], Tiziano Cassina [c], Jens Kuhle [b], Gabriele Casso [c], Pamela Agazzi [a,*]

[a] Neurocenter of Southern Switzerland, Civic Hospital, Lugano, Switzerland
[b] Neurologic Clinic and Policlinic, Departments of Medicine, Biomedicine and Clinical Research, University Hospital Basel, University of Basel, Basel, Switzerland
[c] Cardiocentro Ticino, Lugano, Switzerland

ARTICLE INFO

Article history:
Received 28 May 2019
Revised 8 July 2019
Accepted 8 July 2019
Available online 30 July 2019

ABSTRACT

Functional outcome in patients with postanoxic encephalopathy after cardiac arrest (CA) often remains unclear, and there is a strong need of new prognostication measures. We aimed at investigating serum neurofilament light (NfL) chain concentration in patients with a postanoxic encephalopathy after CA and its prognostic potential.

Serum samples were prospectively collected at different time points after CA in consecutive patients admitted to the intensive care unit (ICU) of Ticino Cardiocentre (Lugano, Switzerland) between June 2017 and March 2018. Serum NfL concentration was measured using a single molecule array (SIMOA) assay. The association of NfL levels with time to return of spontaneous circulation (ROSC), serum neuronal specific enolase (NSE) concentration, time between CA and sample collection, electroencephalogram (EEG) pattern and clinical outcome (death status at one month) were explored.

Fourteen patients experiencing 15 CAs were included in the study (median age = 58 (57–68) years, 8 males). Median serum NfL concentration was 1027.0 (25.5–6033.7) pg/ml. There were positive associations between serum NfL and time to ROSC (rho = 0.60, $p < 0.0001$), NSE concentration (rho = 0.76, $p < 0.0001$), and severity of brain damage as estimated by EEG, with the highest concentrations measured in patients with suppressed electrical activity (14,954.0 [9006.0–25,364.0] pg/ml). Neurofilament light concentration remained high in samples collected up to 17 days after CA. Median NfL levels were higher among dead than alive patients at one month (6401.7 [3768.5–15,573.3] vs 25.5 [25.2–75.4] pg/ml). High NfL levels performed better than NSE in predicting death status at one month (NfL area under the curve (AUC) = 0.98, 95% confidence interval (CI) = 0.94–1.00; NSE AUC = 0.80, 95% CI = 0.67–0.94).

These results support the potential inclusion of serum NfL in the battery of prognostication measures to be used in patients with postanoxic encephalopathy in ICU settings.

This article is part of the Special Issue "Proceedings of the 7th London-Innsbruck Colloquium on Status Epilepticus and Acute Seizures"

1. Introduction

Postanoxic encephalopathy after cardiac arrest (CA) is among the leading causes of mortality and long-term neurological disability in patients admitted to intensive care units (ICU) [1,2]. Several measures are currently used to aid prognosis, including scores based on clinical findings, serum neuronal specific enolase (NSE) concentration, electroencephalogram (EEG), somatosensory evoked potentials, and neuroimaging [3]. Peripheral NSE concentration predicts poor outcome after CA with good sensitivity and specificity. However, only samples taken within 3 days from the event have prognostic value and false positive results can also occur as a result of haemolysis [4].

Serum neurofilament light (NfL) concentration represents a valid biomarker of neuronal degeneration and clinical outcome in several neurological conditions including multiple sclerosis, dementia, amyotrophic lateral sclerosis, and brain trauma [5–8]. A recent study measured serum NfL levels in samples collected in the first 72 h after CA as part of the Target Temperature Management After CA trial, showing a higher predictive potential of poor neurological outcome as compared to currently available prognostication methods (including NSE) [9].

* Corresponding author at: Department of Neurology, Neurocenter of Southern Switzerland, Civic Hospital, Lugano, Switzerland.
E-mail address: pamela.agazzi@eoc.ch (P. Agazzi).

https://doi.org/10.1016/j.yebeh.2019.07.033
1525-5050/

We simultaneously initiated a small prospective pilot study with the aim of: 1) estimating serum NfL concentration in patients with a postanoxic encephalopathy after CA using a single molecule array (SIMOA) assay; 2) investigating the potential relation of serum NfL with time to return of spontaneous circulation (ROSC), time between CA and sample collection, serum NSE concentration, and EEG pattern; and 3) exploring the potential of serum NfL in predicting death at one month after CA as compared to NSE.

2. Methods

We included in the study all patients with a postanoxic encephalopathy due to CA who were consecutively admitted to the ICU of Ticino Cardiocentre (Lugano, Switzerland) between June 2017 and March 2018. All patients underwent a careful neurological examination and serum samples collection for NfL measurements at different time points after CA during hospitalization. Neurofilament light concentration was measured using a SIMOA assay (Quanterix) as previously described [5]. Neuronal specific enolase concentration was also estimated in the same serum samples using an electrochemiluminescence assay (Roche). Standard 10–20 montage EEGs were registered in a minority of patients when needed as determined by ICU specialists and neurologists involved in routine clinical practice, in the absence of sedative drugs [10]. EEG patterns were categorized into: 1) suppressed (either activity < 10 μV in longitudinal montage or burst-suppression patterns); 2) presence of generalized periodic discharges (GPD); and 3) delta–theta coma (background activity with amplitude ≥ 10 μV but frequency < 8 Hz). Only EEGs registered within 48 h of sample collection (before or after) were considered for analyses.

Categorical variables were described by counts and percentages, continuous and ordinal variables by median and interquartile ranges (IQR). Associations between continuous variables were tested using Spearman's correlation. The association between NfL and NSE concentration with death status was tested using univariate and multivariate logistic regression. Prognostic accuracy of NfL and NSE was determined by using receiver operating characteristic (ROC) curves, area under the curve (AUC), and sensitivity and specificity for best cutoffs. All analyses were conducted using the statistical software R and the R packages "ROCR" and "cvAUC" (https://www.r-project.org/).

The study received ethical approval by an independent ethics committee (Comitato Etico Cantonale Bellinzona). A written informed consent was obtained from participants (when possible) or legal representatives of patients.

3. Results

A total of 14 CA patients were included in the study (median age = 58 (57–68) years, 8 males). One patient experienced two CAs (a first out-of-hospital CA and a second in-hospital CA, six days apart), for a total of 15 CAs included in the analysis. Median time to ROSC was 27 (15.5–40.0) minutes. Seven patients were dead at one month, with a median time to death equal to 9.0 (5.5–13.5) days after CA. Among the seven patients who survived, six experienced a full neurological recovery within the first week (Cerebral Performance Category [CPC] 1), while one retained important residual cognitive deficits (CPC 3) [11].

The total number of collected serum samples was 37, with a median time to sample collection of 2 (1–3) days (range 0–17 days) since CA. The overall median serum NfL and NSE concentrations were 1027.0 (25.5–6033.7) pg/ml and 28.7 (14.8–89.7) μg/L. Serum NfL concentration was positively correlated with both time to ROSC (rho = 0.60, p < 0.0001, Fig. 1a) and NSE concentration (rho = 0.76, p < 0.0001, Fig. 1b). Five patients had serum samples collected at 7, 8, 9, 11, and 17 days after CA, respectively. Serum NfL concentration remained high and relatively stable at these later time points (mean variation +18% compared to baseline, Fig. 2a), in contrast with a clear decreasing trend in serum NSE concentration (mean variation −47%, Fig. 2b).

Fourteen EEGs were registered in seven patients within 48 h of sample collection (delta–theta coma n = 5; GPD n = 4; suppressed n = 5). Median serum NfL levels appeared higher in samples paired with suppressed EEGs (14,954.0 [9006.0–25,364.0] pg/ml) than GPD (3613.0 [3436.8–4405.9] pg/ml) and delta–theta coma (2444 [10.9–3280.3] pg/ml) (Fig. 3a).

Median NfL levels were higher among dead than alive patients at one month (6401.7 [3768.5–15,573.3] vs 25.5 [25.2–75.4] pg/ml, respectively; odds ratio (OR) = 1.011, 95% confidence interval (CI) = 1.004–1.019, p = 0.003). Serum NSE levels were also positively associated with risk of death (OR = 1.203, 95% CI = 1.005–1.505, p = 0.044). However, when both NfL and NSE were included in the same regression model, only NfL remained significantly associated with death (NfL: OR = 1.016, 95% CI = 1.002–1.031, p = 0.023; NSE: OR = 0.867, 95% CI = 0.676–1.110, p = 0.258). Neurofilament light performed better than NSE in predicting death (NfL AUC = 0.98, 95CI% = 0.94–1.00; NSE AUC = 0.80, 95% CI = 0.67–0.94, Fig. 3b). A cutoff in NfL of 3436.8 pg/ml provided 83% sensitivity and 100% specificity in death prediction.

One patient experienced two CAs six days apart, with full recovery after the first and unfortunately death following the second one. Accordingly, serum NfL concentration was 53.7 and 66.9 pg/ml following the first CA and raised to 3613.0 and 6033.7 pg/ml following the second CA. Interestingly, the only patient who survived despite considerably

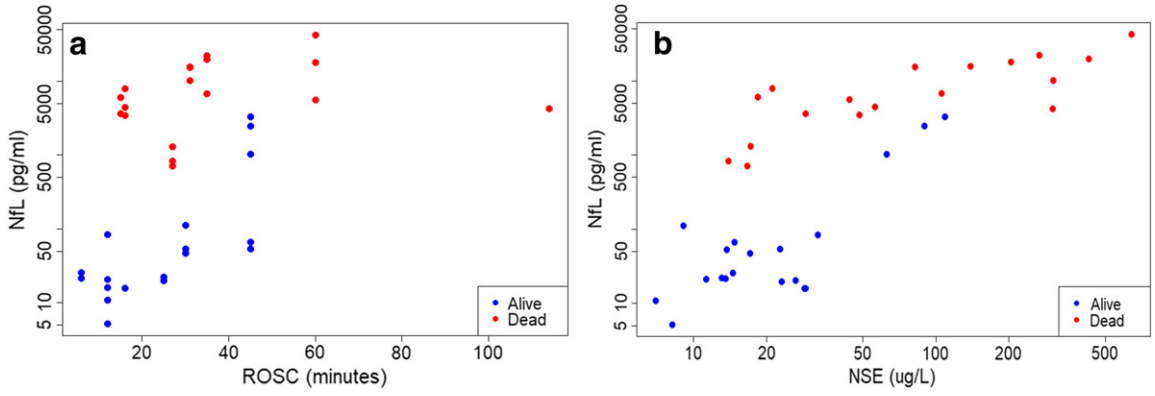

Fig. 1. a) Positive association between serum NfL concentration and time to ROSC (rho = 0.60, p < 0.0001); b) Positive association between serum NfL concentration and NSE concentration (rho = 0.76, p < 0.0001). Each dot represents a separate sample, with clinical outcome also being shown by color (red for death, blue for alive at one month).

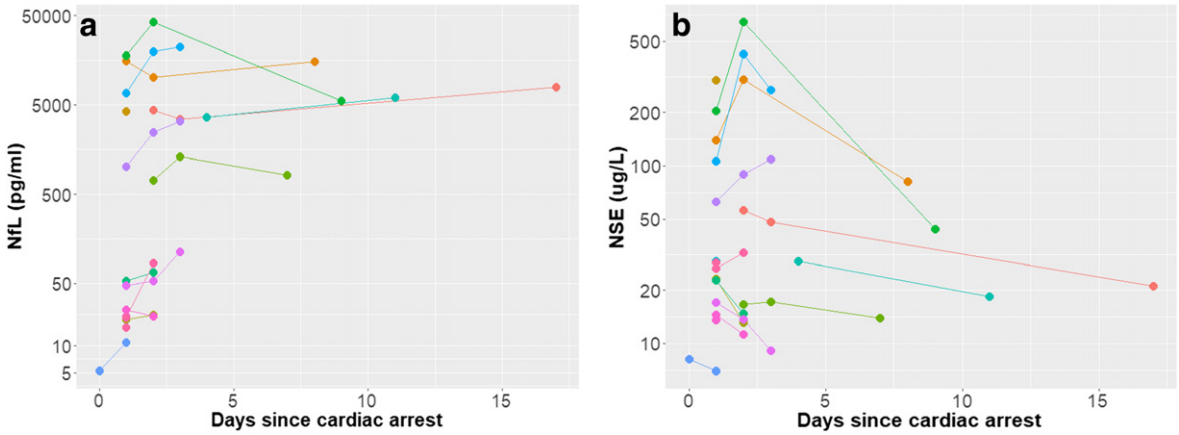

Fig. 2. Temporal relation between days since CA and serum NfL (a) and NSE (b) concentration stratified by patient. Each dot represents a separate sample and individual patients are reported using different colors.

high NfL levels (1027.0–3280.3 pg/ml) was the one with important residual neurological deficits (CPC 3).

4. Discussion

We provide evidence for serum NfL concentration being an accurate marker of neuronal damage in patients with postanoxic encephalopathy due to CA. Serum NfL levels were indeed associated with time to ROSC, i.e., the duration of anoxic insult. Furthermore, high serum NfL concentration was a strong predictor of death in our small cohort of patients, with a better prognostic performance as compared to NSE. Despite the small sample size of our cohort, these results mirror those of the recent investigation by Moseby-Knappe et al. in which more than 700 patients were included, and NfL also performed better than NSE in outcome prognosis [9]. This makes our results unlikely to be due to chance.

As expected, serum NfL and NSE concentrations were also positively correlated with each other. Despite the relatively small number of collected samples, we were able to investigate NSE and NfL at different time points (up to 17 days after CA). Serum NSE levels are known to decrease after the first 72 h since CA, and their value is therefore limited to this initial interval [3,12]. In contrast, serum NfL concentration appeared to remain steadily high in our cohort even at late time points (i.e., >72 h after CA). This has never been shown and suggests the potential use of NfL as both early and late prognostic markers of outcome after CA. Interestingly, the highest serum NfL concentrations were observed in those patients with evidence of suppressed electrical activity on EEG. This finding is also entirely new and suggests the poor outcome seen in patients with suppression and burst-suppression patterns on EEG is directly related to the extent of neuronal death after the anoxic insult [3, 13].

This study has several strengths including its prospective nature, and the inclusion of all consecutive CA patients admitted to the same ICU. Furthermore, we made use of the SIMOA platform which currently represents the assay with the highest sensitivity when measuring NfL concentration [14]. Finally, this is the first study exploring serum NfL levels in samples collected at late time points after CA (i.e., >72 h after CA) and their potential relation to electrical neuronal activity as measured by EEG. Several limitations need to be acknowledged. First, as a pilot study, only a limited number of individuals was included. This recommends caution in the interpretation of the results (in particular sensitivity and specificity measures) and also precluded the possibility to explore the association of NfL with a more precisely defined clinical outcome, e.g., using the CPC scale (rather than death status). Second,

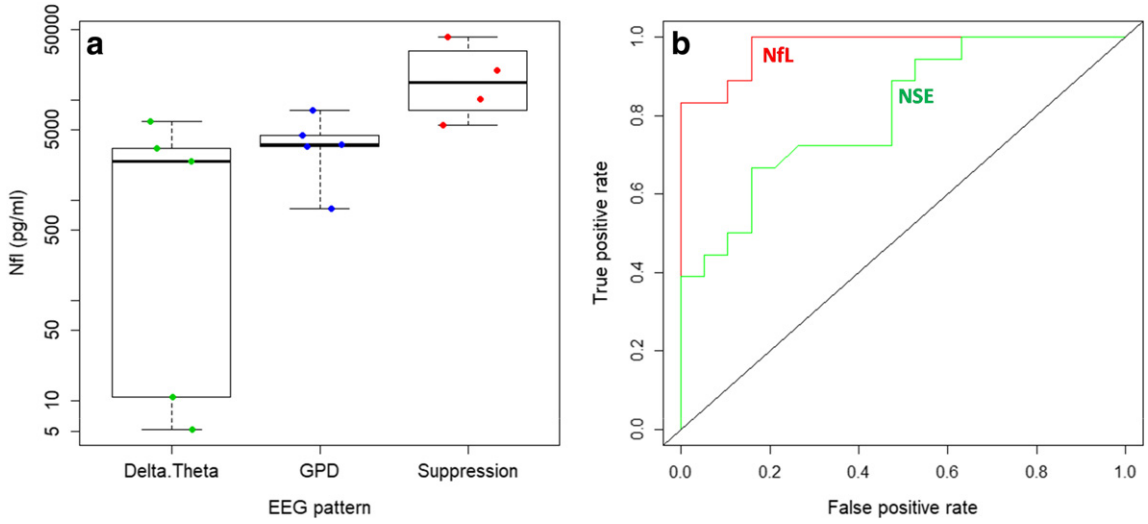

Fig. 3. a) Relation between EEG pattern and serum NfL concentration (higher NfL levels observed in suppressed EEG than in GPD and delta–theta coma); b) Performance of NfL (red) and NSE (green) in death prediction at one month (NfL AUC = 0.98, 95CI% = 0.94–1.00; NSE AUC = 0.80, 95% CI = 0.67–0.94).

sampling time points were not standardized and differed between patients. Additional studies using standardized sampling time points will be needed to precisely define serum NfL kinetics in CA patients. Third, EEG were only performed when needed in clinical practice and were therefore available for a minority of patients. The relation between NfL concentration and additional EEG characteristics (e.g., alpha coma, status epilepticus, and reactivity to stimuli) will need to be investigated in cohorts of larger sample size.

To conclude, we provide additional evidence in support of NfL as a potentially powerful serum biomarker of neuronal damage in patients with postanoxic encephalopathy due to CA, being seemingly associated with time to ROSC, severity of EEG pattern and risk of death, with a prognostication power potentially greater than that of NSE.

Declaration of Competing Interests

None

References

[1] Nielsen N, Friberg H. Temperature management after cardiac arrest. Curr Opin Crit Care 2015. https://doi.org/10.1097/MCC.0000000000000203.
[2] Nolan JP, Ferrando P, Soar J, Benger J, Thomas M, Harrison DA, et al. Increasing survival after admission to UK critical care units following cardiopulmonary resuscitation. Crit Care 2016. https://doi.org/10.1186/s13054-016-1390-6.
[3] Rossetti AO, Rabinstein AA, Oddo M. Neurological prognostication of outcome in patients in coma after cardiac arrest. Lancet Neurol 2016. https://doi.org/10.1016/S1474-4422(16)00015-6.
[4] Ramont L, Thoannes H, Volondat A, Chastang F, Millet MC, Maquart FX. Effects of hemolysis and storage condition on neuron-specific enolase (NSE) in cerebrospinal fluid and serum: implications in clinical practice. Clin Chem Lab Med 2005. https://doi.org/10.1515/CCLM.2005.210.
[5] Disanto G, Barro C, Benkert P, Naegelin Y, Schädelin S, Giardiello A, et al. Serum Neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Ann Neurol 2017. https://doi.org/10.1002/ana.24954.
[6] Rohrer JD, Woollacott IOC, Dick KM, Brotherhood E, Gordon E, Fellows A, et al. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. Neurology 2016. https://doi.org/10.1212/WNL.0000000000003154.
[7] Lu CH, Macdonald-Wallis C, Gray E, Pearce N, Petzold A, Norgren N, et al. Neurofilament light chain: a prognostic biomarker in amyotrophic lateral sclerosis. Neurology 2015. https://doi.org/10.1212/WNL.0000000000001642.
[8] Shahim P, Gren M, Liman V, Andreasson U, Norgren N, Tegner Y, et al. Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. Sci Rep 2016. https://doi.org/10.1038/srep36791.
[9] Moseby-Knappe M, Mattsson N, Nielsen N, Zetterberg H, Blennow K, Dankiewicz J, et al. Serum neurofilament light chain for prognosis of outcome after cardiac arrest. JAMA Neurol 2018. https://doi.org/10.1001/jamaneurol.2018.3223.
[10] Seeck M, Koessler L, Bast T, Leijten F, Michel C, Baumgartner C, et al. The standardized EEG electrode array of the IFCN. Clin Neurophysiol 2017. https://doi.org/10.1016/j.clinph.2017.06.254.
[11] Jennett B, Bond M. Assessment of outcome after severe brain damage. Lancet 1975. https://doi.org/10.1016/S0140-6736(75)92830-5.
[12] Fogel W, Krieger D, Veith M, Adams HP, Hund E, Storch-Hagenlocher B, et al. Serum neuron-specific enolase as early predictor of outcome after cardiac arrest. Crit Care Med 1997. https://doi.org/10.1097/00003246-199707000-00012.
[13] Sivaraju A, Gilmore EJ, Wira CR, Stevens A, Rampal N, Moeller JJ, et al. Prognostication of post-cardiac arrest coma: early clinical and electroencephalographic predictors of outcome. Intensive Care Med 2015. https://doi.org/10.1007/s00134-015-3834-x.
[14] Kuhle J, Barro C, Andreasson U, Derfuss T, Lindberg R, Sandelius Å, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: \ELISA, electrochemiluminescence immunoassay and SIMOA. Clin Chem Lab Med 2016. https://doi.org/10.1515/cclm-2015-1195.