Serum neurofilament measurement improves clinical risk scores for outcome prediction after cardiac arrest: results of a prospective study

Sabina Hunziker1,2,4*, Adrian Quinto2†, Maja Ramin-Wright2†, Christoph Becker2, Katharina Beck2, Alessia Vincent2, Kai Tisljar1, Giulio Disanto4,5, Pascal Benkert6, David Leppert4,5, Hans Pargger1,4, Stephan Marsch1,4, Raoul Sutter1,3,4, Nils Peters4,5 and Jens Kuhle4,5

Abstract

Background: A recent study found serum neurofilament light chain (NfL) levels to be strongly associated with poor neurological outcome in patients after cardiac arrest. Our aim was to confirm these findings in an independent validation study and to investigate whether NfL improves the prognostic value of two cardiac arrest-specific risk scores.

Methods: This prospective, single-center study included 164 consecutive adult after out-of-hospital cardiac arrest (OHCA) patients upon intensive care unit admission. We calculated two clinical risk scores (OHCA, CAHP) and measured NfL on admission within the first 24 h using the single molecule array NF-light® assay. The primary endpoint was neurological outcome at hospital discharge assessed with the cerebral performance category (CPC) score.

Results: Poor neurological outcome (CPC > 3) was found in 60% (98/164) of patients, with 55% (91/164) dying within 30 days of hospitalization. Compared to patients with favorable outcome, NfL was 14-times higher in patients with poor neurological outcome (685 ± 1787 vs. 49 ± 111 pg/mL), with an adjusted odds ratio of 3.4 (95% CI 2.1 to 5.6, p < 0.001) and an area under the curve (AUC) of 0.82. Adding NfL to the clinical risk scores significantly improved discrimination of both the OHCA score (from AUC 0.82 to 0.89, p < 0.001) and CAHP score (from AUC 0.89 to 0.92, p < 0.05). Adding NfL to both scores also resulted in significant improvement in reclassification statistics with a Net Reclassification Index (NRI) of 0.58 (p < 0.001) for OHCA and 0.83 (p < 0.001) for CAHP.

Conclusions: Admission NfL was a strong outcome predictor and significantly improved two clinical risk scores regarding prognostication of neurological outcome in patients after cardiac arrest. When confirmed in future outcome studies, admission NfL should be considered as a standard laboratory measure in the evaluation of OHCA patients.

Keywords: Serum neurofilament, Cardiac arrest, Prognosis, CAHP, OHCA, Cardiopulmonary resuscitation

Introduction

Despite the increased survival rates thanks to medical treatments, the mortality and risk for neurological deficits remains high for cardiac arrest patients [1–3]. Prognostication of outcome upon admission is difficult in these patients, yet, early identification of predictors
for a poor outcome after out-of-hospital cardiac arrest (OHCA) would facilitate the therapeutic management, decision-making, and communication with relatives [4]. The associated high economic burden of comatose critically ill patients further increases the impact not just on an individual level, but also on society at large. For risk prediction of adverse clinical outcome, several clinical tools have been developed. Two scores were specifically developed for cardiac arrest patients including the Out-of-Hospital Cardiac Arrest score (OHCA) and the Cardiac Arrest Hospital Prognosis score (CAHP) [5–7]. These scores, however, still have limited accuracy and, thus, bear the risk of misclassifying patients. The improvement of these clinical scores by the addition of biomarkers reflecting pathophysiologic mechanisms of neural damage is an unmet medical need. Herein, different biomarkers indicating brain injuries after OHCA have been studied [8, 9], yet neuron-specific enolase (NSE) is currently the only biomarker recommended by guidelines as a prognostic blood marker for patients after cardiac arrest [9–12].

Recently, elevated levels of neurofilament light chain (NFL) in blood samples have been established as marker of neuronal damage in traumatic brain injury and many acute and chronic neurologic diseases [13–19]. NFL thus has potential to further improve assessment of neurologic damage in OHCA patients. To date, one pilot study investigated this biomarker in cerebrospinal fluid [20] and a few studies in the blood of patients after cardiac arrest [21–24]. Two studies analyzed neurofilament levels with standard immunoassays and reported associations of NFL with poor neurological outcome in patients after cardiac arrest [21, 22]. One of these studies used an ultrasensitive single molecule array (Simoa) assay to test prognostication of neurological outcome after OHCA in a large cohort of patients [23]. Serum NFL levels at 24 h after cardiac arrest showed high discrimination regarding long-term poor neurological outcome with an area under the curve (AUC) of 0.94–0.95, which was better compared to other biomarkers (i.e., tau, neuron-specific enolase (NSE), and S100). Before wide-spread implementation of NFL, independent validation in a larger sample is mandatory.

Herein, the aim of this study was to externally validate the prognostic accuracy of NFL in a well characterized sample of patients after OHCA and to study whether NFL improves current OHCA specific risk scores, namely, the OHCA and CHAP scores.

Methods

Study setting
This is a prospective observational study including consecutive patients from November 2012 until February 2016 with data obtained in the COMMUNICATE trial at the University Hospital Basel, Switzerland. The main purpose of this study is to investigate novel biomarkers for risk stratification of OHCA patients. The methods used for this study have been published previously [7, 25, 26]. The study was approved by the local ethics committee. Patients or their relatives provided informed consent for study participation.

This manuscript adheres to the STROBE guidelines.

Study population
We included consecutive patients after cardiac arrest who were admitted to the intensive care unit (ICU) of the University Hospital Basel, a Swiss tertiary academic medical center, into the study. We did not use any exclusion criteria regarding the patients’ characteristics and type or duration of the cardiac arrest except for patients having to be adults.

The treatment of patients regarding the cardiac arrest was based on the clinical routine in our ICU without interaction with the research team. Withdrawal of life-sustaining therapy was done per clinical routine after in-depth discussion within the treating team and the patients’ family based on presumed patients’ will, the medical and social situation and the patients prognosis as assessed by all available clinical, neurological and laboratory data (not including levels of NFL).

Data collection
We calculated all scores as suggested [5, 6, 27, 28] and as described in more detail in a previous publication [7]. In brief, we used data collected on the first day of admission. Resuscitation data were collected from clinical records, including no-flow time [time from cardiac arrest to start of basic life support (BLS)], low-flow time [time from start of BLS to return of spontaneous circulation (ROSC)], initial rhythm, setting and location of arrest, epinephrine dose given, as well as information on whether bystanders observed the cardiac arrest and started cardiopulmonary resuscitation (CPR). Clinical parameters [e.g., Glasgow Coma Scale (GCS)], sociodemographics (e.g., age, gender) and comorbidities (i.e., coronary disease, congestive heart failure, hypertension, chronic obstructive pulmonary disease (COPD), malignant disease, diabetes, chronic kidney disease, liver failure) were recorded from medical records or during an interview with patients’ relatives. We also collected initial levels of routine blood markers, (e.g. pH, lactate, creatinine).

We also calculated two risk scores that were specifically developed for outcome prediction in the cardiac arrest patient population including the out-of-hospital cardiac arrest (OHCA) score and the Cardiac Arrest Hospital
30 days after discharge. A further secondary endpoint was all-cause mortality at discharge. We calculated univariable and multivariable logistic regression models to evaluate the association of NfL levels with the primary and secondary endpoints. Data were inspected for normality by use of Q–Q plots. To achieve a normal distribution, data of NfL levels were log transformed with a base of 10. Odds ratios (OR) and 95% confidence intervals (CI) were reported as a measure of association. For regression analysis, we also used quartiles of NfL to study association with outcome. Covariates used in the multivariate analysis were selected based on prior evidence of an association with unfavourable neurological outcome for patients with OHCA. Multivariate models were adjusted for age, gender and comorbidities. In a further step, we additionally included OHCA and CAHP scores (which are based on several prognostic parameters) into the models. In addition to regression analysis to study strength of association, we also calculated area under the ROC curve (AUC) to provide a measure of discrimination for all parameters. To understand whether NfL would improve established risk scores, we also calculated AUCs of combined models (score plus NfL) to see whether NfL would improve discrimination of the scores. We also calculated the Net Reclassification Index (NRI) across risk categories of 5%, 10%, 30%, 50% and 80% and the Integrated Discrimination Index (IDI) as proposed by Pencina and colleagues [35].

We also investigated the prognostic performance of NfL regarding sensitivity, specificity, positive and negative predictive values and likelihood ratios at three cut-offs (25, 50, 75 pg/ml), which were close to the median NfL as well as the lower and upper interquartile range. Additionally, we investigated two cut-offs to maximize specificity and sensitivity. STATA 15.0 was used for all statistical analyses and a two-sided p value of < 0.05 was considered significant.

Results

Baseline characteristics
From the 164 included patients, 98 (60%) had poor neurological outcome and 91 (55%) patients died. The baseline characteristics of the cohort overall and stratified based on neurological outcome are shown in Table 1. Overall, the mean age was 63 years and 28.7% were female. Patients showed relevant comorbidities and cardiovascular risk factors. Patients with poor neurological outcome had less pre-existing coronary heart disease and a higher incidence of diabetes mellitus and malignant disease than patients with good neurological outcome. In patients with poor neurological outcome the cardiac arrest was more often unwitnessed, and they less frequently received bystander CPR and further had longer no-flow and low flow time than patients with a
good neurological outcome. In terms of the initial clinical parameter, patients with poor neurological outcome had a lower GCS, lower pH levels and higher lactate levels than patients with a good neurological outcome.

Primary endpoint: Poor neurological outcome measured with the cerebral performance category (CPC) score

NfL levels were higher in patients with poor neurological outcome compared to patients with good neurological outcome [mean/median 685/116 (SD ± 1787) vs mean/median 49/27 (SD ± 111), \( p = 0.004 \)] with an univariate OR (of log-transformed NfL) of 2.9 (95% CI 2.0 to 4.3,

### Table 1 Baseline characteristics

| Factor                              | CPC All | Good neurological outcome, CPC 1–2 | Poor neurological outcome, CPC 3–5 | \( p \) value |
|-------------------------------------|---------|------------------------------------|-----------------------------------|-------------|
| **N, n (%)**                         | 164 (100%) | 66 (40%)                           | 98 (60%)                          |             |
| **Sociodemographics**               |         |                                    |                                   |             |
| Age, mean (SD)                      | 63 (15) | 60 (17)                            | 65 (14)                           | 0.027       |
| Male, n (%)                         | 117 (71.3%) | 55 (83%)                           | 62 (63%)                          | 0.008       |
| **Comorbidities**                   |         |                                    |                                   |             |
| Coronary heart disease, n (%)       | 109 (66.5%) | 50 (76%)                           | 59 (60%)                          | 0.044       |
| Congestive heart failure, n (%)     | 20 (12.2%) | 7 (11%)                            | 13 (13%)                          | 0.81        |
| COPD, n (%)                         | 15 (9.1%) | 3 (5%)                             | 12 (12%)                          | 0.11        |
| Liver disease, n (%)                | 3 (1.8%) | 0 (0%)                             | 3 (3%)                            | 0.27        |
| Hypertension, n (%)                 | 86 (52.4%) | 37 (56%)                           | 49 (50%)                          | 0.52        |
| Diabetes, n (%)                     | 39 (23.8%) | 9 (14%)                            | 30 (31%)                          | 0.015       |
| Chronic kidney disease, n (%)       | 24 (14.6%) | 8 (12%)                            | 16 (16%)                          | 0.51        |
| Malignant disease, n (%)            | 14 (8.5%) | 2 (3%)                             | 12 (12%)                          | 0.047       |
| Neurological disease, n (%)         | 13 (7.9%) | 2 (3%)                             | 11 (11%)                          | 0.077       |
| **Resuscitation measures**          |         |                                    |                                   |             |
| No-flow time, min, mean (SD)        | 4.22 (5.93) | 1.70 (2.96)                        | 6.02 (6.83)                       | < 0.001     |
| Low-flow time, min, mean (SD)       | 20.42 (15.13) | 16.53 (14.30)                     | 23.12 (15.17)                     | 0.006       |
| **Cardiac arrest setting**          |         |                                    |                                   |             |
| At home                             | 63 (38.4%) | 16 (24%)                           | 47 (48%)                          | < 0.001     |
| In public                           | 78 (47.6%) | 43 (65%)                           | 35 (36%)                          |             |
| In hospital                         | 23 (14.0%) | 7 (11%)                            | 16 (16%)                          |             |
| Witnessed                           | 137 (83.5%) | 63 (95%)                           | 74 (76%)                          | < 0.001     |
| Bystander CPR                       | 103 (62.8%) | 51 (77%)                           | 52 (53%)                          | 0.002       |
| **Initial heart rhythm**            |         |                                    |                                   |             |
| Ventricular tachycardia             | 6 (3.7%) | 2 (3%)                             | 4 (4%)                            | < 0.001     |
| Ventricular fibrillation            | 88 (53.7%) | 53 (80%)                           | 35 (36%)                          |             |
| Asystole                            | 35 (21.3%) | 2 (3%)                             | 33 (34%)                          |             |
| Pulseless electrical activity       | 29 (17.7%) | 6 (9%)                             | 23 (23%)                          |             |
| Unknown                             | 6 (3.7%) | 3 (5%)                             | 3 (3%)                            |             |
| Epinephrine during resuscitation (mg), mean (SD) | 2.18 (2.55) | 1.08 (1.76)                       | 2.96 (2.73)                       | < 0.001     |
| Targeted temperature management (TTM), n (%) | 109 (66.5%) | 40 (61%)                           | 69 (70%)                          | 0.19        |
| **Initial clinical parameter and levels of routine blood markers** |        |                                    |                                   |             |
| GCS, mean (SD)                      | 7.3 (4.1) | 7 (5)                              | 4 (1)                             | < 0.001     |
| pH, mean (SD)                       | 7.25 (0.12) | 7.29 (0.09)                       | 7.22 (0.13)                       | 0.003       |
| Lactate, mean (SD)                  | 7.3 (4.1) | 5.9 (3.3)                          | 8.3 (4.2)                         | < 0.001     |
| NfL (pg/ml), mean (SD)              | 429 (1415) | 49 (111)                           | 685 (1787)                        | 0.004       |
| NfL (pg/ml), median (IQR)           | 51 (21,173) | 27 (13,46)                        | 116 (41,330)                      | < 0.001     |

Data presented as n (%) or mean (standard deviation) and median (Inter Quartile Range, IQR). COPD, chronic obstructive pulmonary disease; CPR, cardiopulmonary resuscitation; NfL, Neurofilament Light Chain; GCS, Glasgow Coma Scale; Neurological disease includes cerebrovascular diseases (e.g., stroke, brain haemorrhage), degenerative diseases such as multiple sclerosis or Parkinson’s disease and peripheral neurological disease.
Table 2 Comparison between scores to predict primary and secondary endpoints

| Quartiles of NFL | Good neurological outcome (CPC 1–2) | Poor neurological outcome (CPC 3–5) | p value | Univariate | Multivariate<sup>a</sup> adjusted | NFL and OHCA score: multivariate<sup>a</sup> adjusted | NFL and CAHP score: multivariate<sup>a</sup> adjusted |
|------------------|----------------------------------|-----------------------------------|---------|------------|---------------------------------|-----------------------------------------------|-----------------------------------------------|
| n = 66           | n = 98                            |                                   |         | OR (95% CI), p | AUC | OR (95% CI), p | AUC combined with NFL | OR (95% CI), p | AUC combined with NFL |
| 1st quartile     | 29 (44%)                         | 12 (12%)                          | <0.001  | 2.93 (2.01, 4.27), p < 0.001 | 0.82 | 3.44 (2.13, 5.55), p < 0.001 | 2.81 (1.69, 4.67), p < 0.001 | 0.89 | 3.45 (1.89, 6.29), p < 0.001 |
| 2nd quartile     | 25 (38%)                         | 16 (16%)                          |         | Reference group | Reference group | Reference group | Reference group | Reference group |
| 3rd quartile     | 10 (15%)                         | 31 (32%)                          | <0.001  | 1.55 (0.62, 3.88), p = 035 | 1.35 (0.46, 4.03), p = 0.59 | 1.02 (0.28, 3.7), p = 0.97 | 1.01 (0.24, 4.23), p = 098 |
| 4th quartile     | 2 (3%)                           | 39 (40%)                          | <0.001  | 7.49 (2.81, 19.96), p < 0001 | 5.59 (1.65, 18.94), p = 0.06 | 3.33 (0.82, 13.41), p = 0.091 | 3.99 (0.84, 18.96), p = 008 |
| OHCA, mean (SD)  | 11 (16)                          | 32 (17)                           | <0.001  | 1.08 (1.05, 1.1), p < 0001 | 1.08 (1.05, 1.1), p < 0001 | 1.07 (1.04, 1.1), p < 0001 | 1.04 (1.02, 1.06), p < 0.001 |
| CAHP, mean (SD)  | 118 (41)                         | 182 (38)                          | <0.001  | 1.04 (1.03, 1.05), p < 0001 | 1.04 (1.03, 1.05), p < 0001 | 1.04 (1.02, 1.06), p < 0001 | 1.04 (1.02, 1.06), p < 0001 |

Primary endpoint: neurological outcome (CPC)

NFL, mean (SD) 49 (111) 685 (1787) 0.004 2.93 (2.01, 4.27), p < 0.001
NFL, median (IQR) 27 (13, 46) 116 (41, 330) 0.004 2.93 (2.01, 4.27), p < 0.001

Quartiles of NFL

Primary endpoint: neurological outcome (CPC)
Table 2 (continued)

| Good neurological outcome (mRs 0–2) | Poor neurological outcome (mRs 3–6) | p value | Univariate | Multivariatea adjusted | NFL and OHCA score: multivariatea adjusted | NFL and CAHP score: multivariatea adjusted |
|-------------------------------------|-------------------------------------|---------|------------|------------------------|---------------------------------------------|--------------------------------------------|
| n = 53                              | n = 111                              |         | OR (95% CI), p | AUC | OR (95% CI), p | AUC combined with NFL | OR (95% CI), p | AUC combined with NFL |
| **Secondary endpoint: Neurological outcome (mRs)** | | | | | | | | |
| NFL, mean (SD) | 51 (123) | 609 (1691) | 0.018 | 2.72 (1.85, 3.98), p < 0.001 | 0.8 | 3.01 (1.88, 4.83), p < 0.001 | 2.47 (1.52, 4.04), p < 0.001 | 0.87 | 2.78 (1.64, 4.72), p < 0.001 |
| NFL, median (IQR) | 21 (12, 40) | 92 (37, 284) | <0.001 | Reference group | Reference group | Reference group | Reference group |
| Quartiles of NFL | | | | | | | | |
| 1st quartile | 26 (49.1%) | 15 (13.5%) | <0.001 | Reference group | Reference group | Reference group | Reference group |
| 2nd quartile | 18 (340%) | 23 (20.7%) | 2.21 (0.91, 5.37), p = 0.078 | 2.11 (0.73, 6.1), p = 0.17 | 1.8 (0.56, 5.83), p = 0.326 | 1.89 (0.55, 6.46), p = 0.309 |
| 3rd quartile | 7 (13.2%) | 34 (30.6%) | 8.42 (3.23, 64.4), p < 0.001 | 6.14 (1.73, 21.8), p = 0.005 | 3.6 (0.9, 14.41), p = 0.07 | 3.97 (0.94, 16.7), p = 0.006 |
| 4th quartile | 2 (3.8%) | 39 (35.1%) | 33.8 (7.13, 160.31), p < 0.001 | 66.08 (9.66, 452.17), p < 0.001 | 42.17 (4.72, 376.98), p = 0.001 | 53.32 (5.52, 514.78), p = 0.001 |
| OHCA, mean (SD) | 11 (16) | 30 (18) | <0.001 | 1.06 (1.04, 1.09), p < 0.001 | 0.79 | 1.07 (1.04, 1.1), p < 0.001 | 1.06 (1.03, 1.08), p < 0.001 |
| CAHP, mean (SD) | 117 (42) | 175 (42) | <0.001 | 1.03 (1.02, 1.04), p < 0.001 | 0.84 | 1.03 (1.02, 1.04), p < 0.001 | 1.03 (1.02, 1.04), p < 0.001 |
Table 2 (continued)

|Survivors| Non-Survivors| p value| Univariate| Age, gender, comorbidities adjusted| NFL and OHCA score: multivariate* adjusted| NFL and CAHP score: multivariate* adjusted|
|---|---|---|---|---|---|---|
|n = 73| n = 91| OR (95% CI), p| AUC| OR (95% CI), p| AUC combined with NFL| OR (95% CI), p| AUC combined with NFL|
|NFL, mean (SD)| 136 (701)| 664 (1763)| 0.017| 2.77 (1.94, 3.95), p < 0.001| 2.97 (1.91, 4.62), p < 0.001| 2.42 (1.54, 3.8), p < 0.001| 2.59 (1.6, 4.2), p < 0.001|
|NFL, median (IQR)| 27 (13, 47)| 128 (45, 330)| <0.001| Reference group| Reference group| Reference group| Reference group|
|Quartiles of NFL| | | | | | | |
|1st quartile| 33 (45%)| 8 (9%)| <0.001| Reference group| Reference group| Reference group| Reference group|
|2nd quartile| 25 (34%)| 16 (18%)| 2.64 (0.98, 7.14), p = 0.056| 2.47 (0.75, 8.1), p = 0.135| 2.29 (0.62, 8.42), p = 0.211| 2.32 (0.58, 9.3), p = 0.0235|
|3rd quartile| 11 (15%)| 30 (33%)| 11.25 (3.99, 31.71), p < 0.001| 7.85 (2.19, 28.09), p = 0.002| 5.49 (1.39, 21.76), p = 0.015| 5.87 (1.35, 25.57), p = 0.0019|
|4th quartile| 4 (5%)| 37 (41%)| 38.16 (10.52, 138.44), p < 0.001| 92.29 (15.16, 561.71), p < 0.001| 49.33 (7.29, 333.79), p < 0.001| 65.12 (8.32, 509.95), p < 0.001|
|OHCA, mean (SD)| 14 (18)| 32 (17)| <0.001| 1.06 (1.04, 1.09), p < 0.001| 1.07 (1.04, 1.1), p < 0.001| 1.05 (1.03, 1.08), p < 0.001| 1.08 (1.06, 1.1), p < 0.001|
|CAHP, mean (SD)| 123 (43)| 182 (38)| <0.001| 1.03 (1.02, 1.04), p < 0.001| 1.03 (1.02, 1.04), p < 0.001| 1.03 (1.02, 1.04), p < 0.001| 1.03 (1.02, 1.04), p < 0.001|

Secondary endpoint: 30-day mortality

Data presented as mean (SD) and median (Inter Quartile Range, IQR); NFL = Neurofilament Light Chain (pg/ml); AUC = area under the curve; CAHP = Cardiac Arrest Hospital prognosis (-score); CPC = cerebral performance category; OHCA = Out-of-Hospital Cardiac Arrest (-score); OR = odds ratio; ROC = receiver operating characteristics curve

* Adjusted for age, gender, comorbidities
and a multivariate OR of 3.4 (95% CI 2.1 to 5.6, \(p < 0.001\)) after adjusting for age, gender and comorbidities (Table 2). Further, the risk for poor neurological outcome increased more than 40-fold for patients in the highest quartile compared to the lowest quartile of NFL concentrations [OR 47.1 (95% CI 9.8 to 227.0), \(p < 0.001\)].

NFL showed a comparable prognostic discrimination with an AUC of 0.82 to both established clinical risk scores. Adding NFL to the risk scores further improved discrimination between good and poor neurological outcome (from AUC 0.82 with OHCA score alone to 0.89 in combination with NFL, \(p < 0.001\), from AUC 0.89 with CAHP score alone to 0.92 in combination, \(p < 0.05\)).

NFL also showed a significant improvement in regard to the NRI of 0.58 (\(p < 0.001\)) for OHCA (among patients with poor outcome, adding NFL increased the risk in the statistical model in 30%, while decreasing the risk in 11%; and among patients with favorable outcome, adding NFL decreased the risk of the model in 54% while increasing it in 15%). For the CAHP score, there was also a strong improvement with an NRI of 0.83 (\(p < 0.001\)) (among patients with poor outcome, adding NFL increased the risk in the statistical model in 36%, while decreasing the risk in 7%; and among patients with favorable outcome, adding NFL decreased the risk of the model in 67% while increasing it in 12%). The IDI for OHCA were 0.15 (\(p < 0.001\)) and for CAHP 0.25 (\(p < 0.001\)).

Table 3 shows sensitivity and specificity, positive and negative predictive value as well as positive and negative likelihood ratios for NFL at three different cut-offs. At the calculated optimal cut-off (Youden-Index) of 50 pg/ml, we found a corresponding sensitivity of 72.4% and specificity of 81.8%, with 85.5% positive predictive value and 66.7% negative predictive value for poor neurological outcome. Further, we calculated prognostic measures based on receiver operating characteristic (ROC) curve analysis for the cut-offs at a NFL level of 25 pg/ml and 75 pg/ml. For the cut-off of 25 pg/ml, the sensitivity was 87.8% and specificity 47%, with 71.1% positive predictive value and 72.1% negative predictive value. Finally, a cut-off of 75 pg/ml revealed a sensitivity of 61.2%, specificity of 90.9%, a 90.9% positive predictive value, and a negative predictive value of 61.2%.

### Table 3 Performance of NFL at different cut-off points to predict neurological Outcome

|                   | CPC score |                   | mRS |                   | Mortality 30 days |
|-------------------|-----------|-------------------|-----|-------------------|------------------|
|                   | NFL cut-off 25 pg/ml | NFL cut-off 50 pg/ml | NFL cut-off 75 pg/ml | NFL cut-off 25 pg/ml | NFL cut-off 50 pg/ml | NFL cut-off 75 pg/ml |
| Poor outcome/total per group | 12/43 | 15/38 | 71/83 | 16/43 | 21/38 | 74/83 | 8/43 | 15/38 | 68/83 |
| Sensitivity Pr(+|A) | 87.80% | 72.40% | 61.20% | 85.60% | 66.70% | 55.00% | 91.20% | 74.70% | 63.70% |
| Specificity Pr(−|N) | 47.00% | 81.80% | 90.90% | 50.90% | 83.00% | 90.60% | 47.90% | 79.50% | 89.00% |
| ROC area (sens. + spec.)/2 | 0.67 | 0.77 | 0.76 | 0.68 | 0.75 | 0.73 | 0.7 | 0.77 | 0.76 |
| Likelihood ratio (+) Pr(−|A)/Pr(+|N) | 1.65 | 3.98 | 6.73 | 1.74 | 3.93 | 5.83 | 1.75 | 3.64 | 5.82 |
| Likelihood ratio (−) Pr(−|A)/Pr(+|N) | 0.26 | 0.34 | 0.43 | 0.28 | 0.4 | 0.5 | 0.18 | 0.32 | 0.41 |
| Odds ratio LR(+)/LR(−) | 6.35 | 11.83 | 15.79 | 6.17 | 9.78 | 11.71 | 9.56 | 11.43 | 14.28 |
| Positive predictive value Pr(−|A) | 71.10% | 85.50% | 90.90% | 78.50% | 89.20% | 92.40% | 68.60% | 81.90% | 87.90% |
| Negative predictive value Pr(−|N) | 72.10% | 66.70% | 61.20% | 62.80% | 54.30% | 49.00% | 81.40% | 71.60% | 66.30% |

Data presented as mean (SD); NFL = Neurofilament Light Chain (pg/ml); CAHP = Cardiac Arrest Hospital prognosis (-score); CPC = cerebral performance category; mRS = modified Rankin Scale; LR+ = positive likelihood ratio; LR− = negative likelihood ratio; NPV = negative predictive value; PPV = positive predictive value
ml. For the cut-off of 25 pg/ml, the calculated sensitivity was 85.6% and the specificity 50.9%, with 78.5% positive predictive value and 62.8% negative predictive value. Further, a cut-off of 75 pg/ml showed a sensitivity of 55%, specificity 90.6%, a 92.4% positive predictive value and a negative predictive value of 49%.

Similarly, NfL was significantly associated with mortality, also after adjusting in the full model [mean/median 664/128 (SD 1763) vs mean/median 136/27 (SD 701), \( p = 0.017 \)] with a good prognostic discrimination (AUC 0.83). Also, we found an improvement of the prognostic value by combination of NfL with the OHCA and the CAHP risk scores (AUC from 0.77 to 0.87 and AUC 0.85 to 0.89 respectively).

At the calculated optimal cut-off of 50 pg/ml, we found a sensitivity of 74.7% and specificity of 79.5%, with 81.9% positive predictive value and 71.6% negative predictive value for poor neurological outcome. For the cut-off of 25 pg/ml, the sensitivity was 91.2% and specificity 47.9%, with 68.6% positive predictive value and 81.4% negative predictive value. Finally, a cut-off of 75 pg/ml revealed a sensitivity of 63.7%, specificity of 89%, an 87.9% positive predictive value, and a negative predictive value of 66.3%. Specificity was further increased to 98.5% at NfL cut-off of 229 pg/ml (corresponding sensitivity 37%). Likewise, sensitivity was 98.0% at NfL cut-off of 13 pg/ml (corresponding specificity 23%).

Figure 1 displays Kaplan–Meier curves for 30-day all-cause mortality based on the three different NfL cut-offs.

We additionally performed subgroup analyses to evaluate differences in associations of NfL with neurological outcome in specific patient groups. Results were also robust in these subgroups for different variables (Fig. 2).

Discussion
In this prospective cohort of OHCA patients, we externally validated previous research and found that serum NfL level is a reliable predictor for poor neurological outcome and in-hospital mortality. Associations of NfL and outcome also remained significant in models adjusted for age, gender, comorbidities and cardiac-arrest specific risk scores. In addition, our data show that NfL significantly improved the prognostic value of two established cardiac arrest specific scoring systems (OHCA and CHAP Score) to predict outcome after cardiac arrest. Results of the previous derivation studies and our prospective validation suggest that serum NfL has high potential to
improve patient care regarding early risk stratification in the population of cardiac arrest patients.

Neurofilaments have emerged in recent years as biofluid markers of neuronal damage which causes the release of these intracellular cytoskeleton proteins into cerebrospinal fluid and blood. Due to the development of ultrasensitive methods such as Simoa, levels are now quantifiable in serum or plasma, allowing longitudinal measurements for monitoring purposes. NfL is currently the most widely used marker among the three sizes of neurofilaments, mainly due to superior assay performance. NfL levels closely reflect the rate and degree of disease acuity in many chronic and acute illnesses of the central nervous system, as well as in traumatic brain injury [13]. In the case of OHCA, NfL could be a synergistic prognostic indicator for poor outcome due to first an increase in its level in the initial period of cardiac-arrest induced anoxic brain injury, and later due to an increase caused by neurologically stunned myocardium which again causes brain injury [36].

### Table: Subgroup Analysis of NfL for Primary Endpoint (Neurological Outcome)

| Factor                                      | Odds Ratio (95% CI) | p of interaction |
|---------------------------------------------|---------------------|------------------|
| Overall                                     | 2.93 (2.01, 4.27)   | <0.001           |
| **Gender**                                  |                     |                  |
| Male                                        | 2.96 (1.91, 4.56)   | 0.87             |
| Female                                      | 2.74 (1.21, 6.21)   |                  |
| **Age**                                     |                     |                  |
| Age < 55                                    | 3.90 (1.51, 10.10)  |                  |
| Age 55-65                                    | 2.45 (1.30, 4.63)   |                  |
| Age 65-75                                    | 3.53 (1.44, 8.66)   |                  |
| Age > 75                                    | 3.71 (1.29, 10.73)  |                  |
| **REA circumstances**                       |                     |                  |
| Bystander CPR                                | 3.63 (2.14, 6.14)   | 0.22             |
| No bystander CPR                             | 2.24 (1.28, 3.91)   |                  |
| Onset of cardiac arrest at home              | 5.12 (1.92, 13.71)  |                  |
| Onset of cardiac arrest in public            | 2.30 (1.41, 3.75)   | 0.90             |
| Onset of cardiac arrest in hospital          | 5.12 (1.10, 24.80)  |                  |
| **Therapy**                                  |                     |                  |
| TTM yes                                     | 2.33 (1.55, 3.49)   | 0.03             |
| TTM no                                      | 8.89 (2.85, 27.71)  |                  |
| **Comorbidities**                           |                     |                  |
| Chronic kidney disease (yes)                 | 2.04 (0.80, 5.33)   | 0.29             |
| Chronic kidney disease (no)                  | 3.65 (2.25, 5.92)   |                  |
| Coronary heart disease (yes)                 | 2.47 (1.63, 3.76)   | 0.13             |
| Coronary heart disease (no)                  | 6.39 (2.02, 20.23)  |                  |
| Congestive heart failure (yes)               | 3.33 (0.88, 12.65)  | 0.84             |
| Congestive heart failure (no)                | 2.89 (1.95, 4.29)   |                  |
| COPD (yes)                                   | 6.13 (0.83, 45.31)  | 0.44             |
| COPD (no)                                    | 2.77 (1.89, 4.05)   |                  |
| Neurological disease (yes)                   | 2.59 (0.45, 14.96)  | 0.90             |
| Neurological disease (no)                    | 2.91 (1.97, 4.28)   |                  |
| Malignant disease (yes)                      | 2.45 (0.52, 11.54)  | 0.85             |
| Malignant disease (no)                       | 2.87 (1.94, 4.24)   |                  |
| Diabetes (yes)                               | 4.34 (1.29, 14.61)  | 0.45             |
| Diabetes (no)                                | 2.66 (1.78, 3.96)   |                  |

*Fig. 2* Subgroup analysis of NfL for primary endpoint (neurological outcome). Data is presented as univariable odds ratio (OR) and 95% confidence interval (95% CI). NfL: Neurofilament Light Chain; TTM: Targeted Temperature Management, COPD: Chronic obstructive pulmonary disease.
addition, lifestyle and cardiovascular health have been shown to influence NFL baseline levels and could thus help to identify a high-risk group of patients regarding cardiovascular outcome [37]. Further, in many of these conditions NFL levels are now established on the group level as predictors for later functional neurological and neuropsychological outcome. Importantly, despite these promising results in other neurological illnesses, there has been relatively little clinical data regarding NFL in OHCA patients.

Our results are in line with two previous studies indicating that NFL is a good novel marker for prognosticating neurological outcome after cardiac arrest [21, 23]. While these studies focused on long-term outcomes after six months, our data suggest that NFL also helps to predict short-term clinical outcomes and thus may help navigate the therapeutic management in an early stage after cardiac arrest. We also investigated the potential of NFL to improve the cardiac specific OHCA and the CAHP scores [5, 25, 29]. Importantly, our data show that NFL measured early in the course after cardiac arrest further improves the discrimination of these clinical risk scores. An important advantage of these scores is that their calculation is based on initial ICU parameters that are readily available upon patient admission, allowing to support clinical decision-making regarding the initial management of OHCA patients. At this time, prognostication is particularly challenging as patients are often unconscious, limiting the clinical assessment. Early prognostic information, however, may help to inform relatives about expected risks and thus influence discussions about potential withdrawal of therapy. Herein, providing prognostic information with a high specificity regarding death and/or poor neurological outcome is of high importance as such decisions have huge consequences. Combining prognostic information from both, clinical scores and specific biomarkers may further improve prognostication and risk stratification. Such a strategy may further improve the current recommendation of taking a multimodal approach and not prognosticating patients before 72–96 h. Clearly, interventional research is needed to understand how prognostic information influences these decisions in more detail.

In patients after cardiac arrest, NSE is currently the only biomarker recommended for prognostication by European and American guidelines [38–40]. In a previous study, we found that NSE is highly predictive for adverse outcome when measured at day 3 of admission, while initial NSE levels measured on day 1 provided only little prognostic information [41]. The current analysis as well as another recent study found that the prognostic value of NSE on admission was inferior to NFL indicating that NFL may be a more accurate outcome marker early in the course of disease [23]. This may be explained by differences in marker kinetics and other factors such as influence of hemolysis on measurement characteristics. Importantly, admission NFL had a better prognostic performance to predict outcome compared to NSE.
Thus, future guidelines should consider including NfL for early prognostication in patients after cardiac arrest as NSE shows only suboptimal results in the early course of disease.

In our study, NfL was measured at baseline within 24 h after onset of cardiac arrest. Literature regarding the kinetics of serum NfL after cardiac arrest is still sparse, especially within the early course before 24 h or after 72 h. In previous stroke and cardiac arrest populations, NfL levels in patients with poor outcome were nearly doubled from 24 to 48 h after cardiac arrest, reaching a steady state between 48 and 72 h [19, 23]. As a limitation, we did not measure NfL at other time points than admission, but possibly looking at kinetics could further improve its prognostic value. Importantly, NfL levels may increase after different types of central nervous system and peripheral nervous system injuries and are thus not specific for cardiac arrest. We only assessed neurological comorbidities as an overall item without specifying these in more detail. However, when adjusting for neurological comorbidities, result stayed robust suggesting no confounding in this regard. Further we did not find an effect modification by neurological comorbidities.

**Strength and limitations**

Strengths of this study are the representative sample size with prospective and consecutive inclusion of patients and blinded analysis of blood markers. There are, however, some limitations: First, this is an observational study and is thus only hypothesis generating. We also did not have data on more advanced neurological examinations (e.g., EEG, SSEP) and on cause of death (i.e., brain injury vs. other causes) which would have been interesting regarding NfL performance. Also, no information regarding falls and brain/head trauma was available, which could influence NfL results. Second, we did not measure NfL during follow-up and thus cannot make any statements about its kinetics. Third, the time interval between ROSC and collection of NfL measurements has not been recorded. This measurement would have been valuable to study the dynamics over the first 24 h after ICU admission of NfL levels in our patient group. Forth, we had an important overlap of patients with unfavorable outcome and non-survivors limiting the interpretation of results. Fifth, we did not differentiate the cause of death (i.e., withdrawal of therapy vs. re-arrest or complications). Still, usually, withdrawal of therapy would be expected to occur later in course after rewarining of the patient and discussion with the family, and not within the first 24 h. Also, while prognostic information regarding routine parameters were available to physicians and may have influenced withdrawal decisions, biomarker levels of NfL as well as OHCA and CAHP scores are not part of our routine care and were thus not routinely available to the treating team and did thus not influence decisions. Finally, external validation of the NfL cutoff levels proposed by our analysis is necessary before wide-spread use in clinical practice.

**Conclusions**

Admission NfL was a strong outcome predictor and significantly improved two clinical risk scores regarding prognostication of neurological outcome in patients after cardiac arrest. When confirmed in future outcome studies, admission NfL should be considered as a standard laboratory measures in the evaluation of OHCA patients.

**Abbreviations**

AUC: Area under the curve; BLS: Basic life support; CAHP: Cardiac Arrest Hospital Prognosis; CI: Confidence interval; COPD: Chronic obstructive pulmonary disease; CPC: Cerebral performance category; CPR: Cardiopulmonary resuscitation; GCS: Glasgow Coma Scale; ICU: Intensive care; IQR: Inter Quartile Range; mRS: Modified Rankin Scale; NfL: Neurofilament light chain; NPV: Negative predictive value; NSE: Neuron-specific enolase; OHCA: Out-of-hospital cardiac arrest; OR: Odds ratio; PPV: Positive predictive value; ROC: Receiver-operating characteristic; ROSC: Return of spontaneous circulation; SD: Standard deviation.

**Acknowledgements**

We would like to acknowledge the medical ICU and laboratory staff of the University Hospital of Basel for making this study possible. We thank all patients and their family members for participating in this study.

**Authors’ contributions**

SH conceived the design of the study and und wrote the study proposal. KB, AV, CB, KT, RS, HP, SM and SH collected the data and contributed to the evaluation and interpretation of data. SH, A-Q, and M.R.W. performed statistical analysis of all data and wrote the first draft of the manuscript. JK, G.D., PB, DL, NP, and their team performed the laboratory analyses, and contributed to interpretation of data and writing the manuscript. All authors critically reviewed and approved the final manuscript.

**Funding**

Sabina Hunziker and her research team is supported by the Swiss National Foundation (SNF) (Ref 10001C_192850/1 and 10531C_182422) and the Gottfried Julia Bangerter-Rhyner Foundation (8472/HEG-DSV) and the Swiss Society of General Internal Medicine (SSGIM). Jens Kuhle is supported by the Swiss National Research Foundation (320030_189140/1) and the Gottfried Julia Bangerter-Rhyner Foundation (8472/HEG-DSV) and the Swiss Society of General Internal Medicine (SSGIM). Jens Kuhle is supported by the Swiss National Research Foundation (320030_189140/1).

**Availability of supporting data**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

Our study complies with the Declaration of Helsinki. The Ethics Committee of Northwest and Central Switzerland (Ethikkommission Nordwest- und Zentralschweiz, EKNZ) approved this study.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

1 Intensive Care Unit, University Hospital Basel, University of Basel, Petersgraben 4, 4031 Basel, Switzerland. 2 Medical Communication and Psychosomatic Medicine, University Hospital Basel, Klingelbergstrasse 23, 4031 Basel,
Switzerland. 1 Division of Clinical Neurophysiology, Department of Neurology, University Hospital Basel, Petersgraben 4, 4031 Basel, Switzerland. 2 Medical Faculty, University of Basel, Klingelbergstrasse 61, 4056 Basel, Switzerland. 3 Neurologic Clinic and Polyclinic, MS Center and Research Center for Clinical Neuromunnoendocrinology and Neuroscience Basle (PC2NBS), University Hospital Basel, University of Basel, Basel, Switzerland. 4 Clinical Trial Unit Basel, Department of Clinical Research, University Hospital Basel, University of Basel, Basel, Switzerland.

Received: 10 October 2020  Accepted: 4 January 2021
Published online: 20 January 2021

References

1. Monsieurs KG, Nolan JP, Bossaert LL, Greif R, Maconochie IK, Nikolaou NI, Perkins GD, Soar J, Tru华北 A, Wille J, et al. European Resuscitation council guidelines for resuscitation 2015: section 1. Executive summary. Resuscitation. 2015:95:1–80.

2. Young GB. Clinical practice. Neurologic prognosis after cardiac arrest. N Engl J Med. 2009;361(6):605–11.

3. Blom MT, Beesems SG, Homma PC, Zijlstra JA, Hulleman M, van Hoeijen DA, Barda A, Tijsen JG, Tan HL, Koster RW. Improved survival after out-of-hospital cardiac arrest and use of automated external defibrillators. Circulation. 2014;130(21):1968–76.

4. Nolan JP, Soar J, Cronberg T, Moulaert VR, Deakin CD, Bottiger BW, Friberg H, Sunde K, Sandroni C, et al. European Resuscitation Council and European Society of Intensive Care Medicine 2015 guidelines for post-resuscitation care. Intensive Care Med. 2015;41(12):2039–56.

5. Adrie C, Cariou A, Mouvillier B, Laurent I, Dabbane H, Hantala F, Rhaoui A, Thaungr M, Monch M. Predicting survival with good neurological recovery at hospital admission after successful resuscitation of out-of-hospital cardiac arrest: the OHCA score. Eur Heart J. 2006;27(23):2840–5.

6. Maupain C, Bougouin W, Lamhaut L, Deye N, Diehl JL, Geri G, Perier MC, Romsperg L, Bloum W, Kerkstra M, et al. The CAHPS (Cardiac Arrest Hospital Prognosis) score: a tool for risk stratification after out-of-hospital cardiac arrest. Eur Heart J. 2016;37(12):2043–50.

7. Isenschmid C, Luescher T, Rasiah R, Kalt J, Tondorff T, Gamp M, Becker C, Tislar J, Stutter R, Schuetz P, et al. Performance of clinical risk scores to predict mortality and neurological outcome in cardiac arrest patients. Resuscitation. 2019;136:21–9.

8. Rossetti AO, Rabinstein AA, Oddo M. Neurological prognostication of outcome in patients in coma after cardiac arrest. Lancet Neurol. 2016;15(6):597–609.

9. Stammert P, Collignon O, Hassager C, Wise MP, Hovdenes J, Aasland OC, Waage A, Venugopalan J, Steen H, et al. Cerebrospinal fluid biomarkers for prediction of neurological outcome after out-of-hospital cardiac arrest: the OHCA score. JAMA Neurol. 2018;75(10):1232–7.

10. Steinberg DS, Perler BC, Sechtem U, Desmet W, Fillinger M, et al. Neuromonitoring and neuroprognosis in literature review. JAMA Neurol. 2016;73(5):516–27.

11. Martinell L, Nielsen N, Herlitz J, Karlsson T, Lehn J, Wyllie J, et al. European Resuscitation Council and European Society of Intensive Care Medicine 2015 guidelines for post-resuscitation care. Intensive Care Med. 2015;41(12):2039–56.

12. Tiedt S, Duerer M, Barro C, Kaya AG, Boeck J, Bode FJ, Klein M, Dorm G, Gesienich B, Kellert L, et al. Serum neurofilament light: a biomarker of neuroaxonal injury after ischemic stroke. Neurology. 2018;91(4):e1338–47.

13. Krueger A, Jorres A, Storm C. Mild therapeutic hypothermia alters neuron-specific enolase as an outcome predictor after cardiac arrest. Crit Care. 2009;13(6):R45.

14. Metzker K, Gamp M, Tondorff T, Becker C, Tislar J, Locher S, Schuetz P, Manch S, Hunziker S. Routine blood markers from different biological pathways improve early risk stratification in cardiac arrest patients: Results from a prospective, observational COMMUNICATE study. Resuscitation. 2018;130:138–45.

15. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med. 1985;13(10):818–29.

16. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA. 1993;270(24):2957–63.

17. Hunziker S, Bivens MJ, Cocchi MN, Miller J, Salciccioli J, Howell MD, Donnio MW. International validation of the out-of-hospital cardiac arrest score in the United States. Crit Care Med. 2011;39(7):1670–4.

18. Martinell L, Nielsen N, Herlitz J, Karlsson T, Horn J, Wise MP, Unden J, Rylander C. Early predictors of poor outcome after out-of-hospital cardiac arrest. Crit Care. 2017;21(1):96.

19. Dorn F, Gesierich B, Kellert L, et al. Serum neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Ann Neurol. 2018;83(2):167–70.

20. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008;27(23):157–72 (discussion 207–112).

21. D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008;27(23):157–72 (discussion 207–112).

22. Annals of Neurology. 2012;71(4):530–3.
36. Kenigsberg BB, Barnett CF, Mai JC, Chang JJ. Neurogenic stunned myocardium in severe neurological injury. Curr Neurol Neurosci Rep. 2019;19(11):90.

37. Korley FK, Goldstick J, Mastali M, Van Eyk JE, Barsan W, Meurer WJ, Sussman J, Falk H, Levine D. Serum NFL (neurofilament light chain) levels and incident stroke in adults with diabetes mellitus. Stroke. 2019;50(7):1669–75.

38. Callaway CW, Donnino MW, Fink EL, Geocadin RG, Golan E, Kern KB, Leary M, Meurer WJ, Peberdy MA, Thompson TM, et al. Part 8: post-cardiac arrest care: 2015 American Heart Association guidelines update for cardiopulmonary resuscitation and emergency cardiovascular care. Circulation. 2015;132(18 Suppl 2):S465–482.

39. Nolan JP, Cariou A. Post-resuscitation care: ERC-ESICM guidelines 2015. Intensive Care Med. 2015;41(12):2204–6.

40. Wihersaari L, Tiainen M, Skrifvars MB, Bendel S, Kaukonen KM, Vaahersalo J, Rompapanen J, Pettila V, Reinikainen M. Usefulness of neuron specific enolase in prognostication after cardiac arrest: Impact of age and time to ROSC. Resuscitation. 2019;139:214–21.

41. Luescher T, Mueller J, Isenschmid C, Kalt J, Rasiah R, Tondorf T, Gamp M, Becker C, Sutter R, Tisljar K, et al. Neuron-specific enolase (NSE) improves clinical risk scores for prediction of neurological outcome and death in cardiac arrest patients: results from a prospective trial. Resuscitation. 2019;142:50–60.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.