Author's response to reviews

Title: Serum neuron-specific enolase as predictor of outcome in comatose cardiac arrest survivors: a prospective cohort study

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Author's response to reviews: see over
Dear Editor,

Thank you for carrying out a careful and informative review of our above referenced manuscript. Please find our responses below. We would like to take this opportunity to thank the reviewers for their careful reading, outstanding comments, suggestions and support, as we believe the revisions clearly improved the revised manuscript.

Please do not hesitate to contact me if additional information is required,

I’m looking forward to hearing from you,

Sincerely,

Cédric Daubin
Corresponding author

Response to Reviewers

Reviewer 1: Johann Reisinger

The major drawback of this study is the fact that three different diagnostic modalities (SSEP, NSE, EEG) were not tested in an independent but in an interdependent manner, because SSEP and EEG findings were used as criteria to terminate intensive care as early as 24-36 hours and 72 hours after cardiac arrest, respectively. Therefore, the reported predictive values of the NSE results (the primary research question of this study) cannot be regarded as completely unbiased.

We agree with this comment and this point is underlined in the Limitations section, point “thirdly”. However, this point is not specific to our paper. In addition, considering the poor neurological prognosis of comatose patients after cardiac arrest, the debate over whether to continue or withdraw intensive care cannot be avoided for the more severe patients for whom intensive treatment could be futile. For this reason, in our paper (see the Treatment and treatment restriction paragraph in the Methods section), we chose to provide criteria for which a decision to withdraw treatment was used for debate rather than falling into the common practice of not mentioning it.

However, this study shows that even patients with peak NSE values reaching almost 97 ng/L (using the Roche assay) may regain consciousness, which is a very important piece of information contradicting the widely published cut-off value of 33 ng/mL. This is the most important message of this investigation. These findings confirm that each commercially available NSE test has to be validated separately for the neurological prognosis of patients after cardiac arrest.
Consequently, results obtained by assays from different manufactures are not interchangeable.

We thank this Reviewer for underlining this important result which is now included in the Conclusion section.

Major Compulsory Revisions:

1. As the authors stated, the purpose of this study was to assess the capability of NSE to predict poor neurological outcome “with certainty”. With a very limited sample size of 97 patients this purpose is obviously an impossible task.

We agree with this comment. Nevertheless, small studies can be combined in meta-analysis, if published.

2. A 95% CI for the proposed NSE cut-off (97 ng/ml) should be given.

Agreed.
This point has now been added.

3. Page 4, Serum NSE: The authors should mention that haemolytic samples were strictly discarded.

Agreed. All samples with visible haemolysis were discarded to avoid any falsely elevated values for serum NSE.
This point has now been added in Methods section

4. Page 5, paragraph on EEG: Was a burst-suppression pattern categorised as malignant in any case irrespective of the level of sedation?

We thank this reviewer for addressing this important point because the conditions of EEG recording are rarely reported in this setting in the literature. For this reason, we chose to report the number of patients still affected by sedative medication at the time of EEG recording, as mentioned in Table 2. Whether sedation may affect EEG results in our study cannot be excluded. However, in our clinic, we pay great attention to the level of sedation: (1) they are monitored according to our local policy (based on the RASS score); (2) sedation is stopped shortly before EEG recording, if possible; (3) expert neurophysiologists are informed of the presence of such drugs to allow better interpretation of EEG findings.

5. SSEPs were performed as soon as possible after the first 24 hours after resuscitation. Were these measurements in patients undergoing mild hypothermia done after restoration of normothermia? Additionally, please specify the time delay of the SSEP measurements after cardiac arrest (median, IQR) in the Results section.
For patients who underwent cooling after resuscitation, clinical and neurophysiological tests, including EEG and SSEP recordings, were performed after warming, as mentioned in the Data collection section in the Methods section. This point has been clarified.

The majority of SSEPs are performed between 24 and 72 hours after cardiac arrest and in all cases during the first week after resuscitation. Unfortunately, the time delay of the SSEP measurements after cardiac arrest is not available.

6. Page 6, second paragraph: This section seems a little bit unclear to me. Standard intensive care management “including induced hypothermia” was obviously not used in all patients but only in a fraction. What is exactly meant by the sentence …left to the discretion of the physician “for the other causes”? These points are now clarified in this paragraph.

The decision to withdraw treatment as early as 72 hours seems a bit early to me. The authors should please discuss that this kind of patient management is not universally accepted. Given the uncertainties of prognostication a minimal time period of 7 days would be more appropriate.

To our knowledge, no recommended delay exists to be used for debating decisions to withdraw treatment in comatose patients after cardiac arrest. Therefore, we provided criteria based on a multimodal approach, from which decisions to withdraw treatment was debated after five days of follow-up (see Treatment and treatment restriction paragraph in the Methods section), and in our practice, only an absence of N20 responses to SSEPS (a result recognized to be the most accurate predictor of an unfavorable outcome) could lead to a decision to discontinue intensive care before day 5. This patient management is already briefly discussed (third point) in the Limitations section of the Discussion section and is in accordance with AAN guidelines. In addition, in our cohort, all patients with unfavorable SSEPs had a mean of 3+/- 1 of predefined pejorative clinical EEG criteria. In this context, it could be ethically questionable to continue intensive treatment for more than 7 days. However, to take into account this reviewer’s comment, we decided to include these last data in the Results section page 9, and in the Discussion section page 10 and page 11 and to let the reader decide on his/her own opinion regarding our treatment restriction policy.

7. How many patients had a DNR order at any time during their stay in the ICU?

Unfortunately, the number of patients with a DNR order was not recorded.

8. Statistical Analysis Section: Thresholds for NSE are given for “the highest specificity and sensitivity, especially for the best specificity”…This sentence should be clarified. The best compromise between sensitivity and specificity...
corresponds to the highest Youden-index, but the best specificity for the purpose of this study is obviously 100%.

Agreed. This point is now clarified in this paragraph: “Thresholds for NSE are given for 100% specificity and the highest sensitivity.”

9. Results Section, page 7, first paragraph: The authors should please provide data on the duration of full intensive care support in patients with unfavourable SSEP results and in those with favourable SSEP results.

The duration of full intensive care support is not available. However, we did provide the survival median time among patients for whom a decision to withdraw active treatment was taken (n=67) overall and according to SSEP results (pejorative, n=45 and not pejorative, n=22).

10. Results Section, page 7, second paragraph: How many patients had NSE measurements at 24 hours and at 72 hours, respectively?

These data have now been added in the Results section.

11. To clarify the possible influence of mild hypothermia on the NSE results, data should be presented separately for patients with and those without hypothermia treatment regarding peak NSE values in patients with CPC 1-3. Additionally, it would be very helpful for the reader to have a separate figure, showing the frequency of different neurological outcome categories in relation to the individual peak NSE concentration (for example like Fig. 3 in Ref. 19) presented separately for patients with and those without hypothermia treatment. Moreover, this kind of separate presentation applies also to the ROC curve (using two curves instead of just one in Figure 2). These changes would considerably improve this manuscript.

We did perform the subgroup analysis you recommended. Keeping in mind the small sample size of the overall cohort (refer to your point 1), we finally decided not to present those subgroups because n=32 for the subgroup without hypothermia. Nevertheless, we provide the highest measurements of NSE in each subgroup of patients CPC 1-3 and CPC 4-5 treated with or without hypothermia, separately in the revised manuscript in Results section page 8. In addition, as suggested by this reviewer (thank you), we provide a figure (named Figure 3 in the revised manuscript) showing the frequency of different neurological outcome categories in relation to the individual peak NSE concentrations of our patients.

12. Regarding the NSE cut-off value, a 95% CI should be given for the positive predictive value of 100%.
Agreed. This point has now been added.

13. Table 2: The inclusion of “No SSEP (N20) bilaterally” as a predictor of poor neurological outcome in this study is highly questionable, because the false positive rate of 0% is not a genuine result of this study, but a logical consequence of the study design, namely to terminate treatment in patients with absent N20 bilaterally very early. The same logic applies to Figure 3.

Correct. SSEP results are now deleted from Table 2 and Figure 3, and SSEP results are presented separately in the last paragraph in the Results section.

14. Table 3: In hospital CPR (%) is not correctly cited for Ref. 19. As stated in this publication, this fraction was 77/177 patients (= 43.5%).

We thank this Reviewer for seeing this. This point has been corrected.

Minor Essential Revisions:
1. Abstract: Conclusion: “Positive” predictive value ...

Thank you for seeing this. This point has been corrected.

2. The term “extra-renal support” should better be replaced by “renal replacement therapy”

Agreed. This point has been corrected in the revised manuscript.

3. Table 1: Medical history: “arrhythmic” instead of “rhythmic”?

Agreed. This point has been corrected.

4. Resuscitation “time” n (%): How does this fit?

This point has been clarified in Table 1 and the term “Resuscitation time” has been changed to “Factors to resuscitation”.

5. The line drawing in Figure 2 is suboptimal. Additionally, at least a few different NSE cut off values should be inserted into this figure.

Correct. Different NSE cut-offs have now been inserted into this figure.
Discretionary Revisions:
None.

Reviewer 2: Hans Friberg

This paper has several good qualifications and will merit for publication but several major issues must be addressed. First, the title is relevant, this paper deals mainly with NSE and how it can be used for prognostication after cardiac arrest. The main message of this study is that:
• The highest NSE (24-72 h after cardiac arrest) can be used as a basis for a decision on level of care with after cardiac arrest
• If a combination of EEG, SSEP, NSE and clinical neurological examination is used, the number of patients with a poor outcome (CPC 4-5) can be increased.
• No tests predicted a good outcome

The patient cohort is indeed mixed, including patients with cardiac arrest of cardiac and non-cardiac origin, in-hospital and out-of-hospital cardiac arrest and even patients who have been treated with induced hypothermia and those who have not. This is good since it increases the generalisability of the use of NSE in the ICU setting.

We thank this Reviewer for his general comments.

The only hesitation I have is weather patients with and without induced hypothermia should be included, since the question weather lowering of temperature affects the cut-off levels of NSE or not is yet not solved. My view, however, is that the same NSE cut-off values may be used, which is supported by the current study. This issue should be more clearly addressed and the results presented separately and discussed.

This point was already discussed by Reviewer Johann Reisinger. Please refer to response 11, Reviewer Johann Reisinger. Also, our sample size does not allow us to perform many subgroup analyses. We now provide the highest measurements of NSE in each subgroups of patients CPC 1-3 and CPC 4-5 treated with or without separately in the revised manuscript.

One major concern is the definitions of good and poor outcome in this study or rather how this issues are addressed. The CPC-scale is the gold standard when reporting outcome after cardiac arrest and a CPC of 1-2 is generally considered a good outcome which is relevant. A poor outcome of 3-5 is generally considered a poor outcome which is relevant as well. However, if biomarkers are to be used as a basis for decision-making of weather or not active care of a patient in coma should continue in the ICU, a cut-off value must indeed correlate with a CPC of 4-5. This must be described better, especially in Table 3, where a poor outcome is
defined differently in different studies which may have to do with different aims of the studies (major compulsory revisions).

We thank this reviewer for supporting our choice of CPC 4-5 as the definition of a poor outcome in this setting. A comment is now added in the Discussion section page 11. In addition “poor outcome” was changed for “poor outcome definition, and number” in Table 3.

Table 3 is a nice overview of published studies in the field but the perspective described above must be added and explained in the text and in the table itself. The very important information on what method was used in each individual study would add significant value to table 3, please change accordingly.

To our knowledge, all studies reported in Table 3 examine the prognostic value of NSE for early prediction of poor outcome in patients after cardiac arrest in the goal to assess the capability of serum NSE to be added to the decision-making process on the continuation or not of intensive care in these patients. This point is clarified in the Discussion section, page 11. In addition, the method used for NSE measurement in each study is now added in Table 3.

Major compulsory revisions:

1 The patient cohort which is studied is actually patients who are alive in the ICU and who remain in coma at 48 hours after cardiac arrest, this could be more clearly stated.

This point is now clarified

2 Some of these 97 patients have a late awakening and have a CPC of 1-3 (26%) 3 months later. My strong suggestion is that NSE at 72 hours be used and analyzed rather than the ”highest observed NSE”. Other studies have shown that the 24-hour values of NSE have a greater variation than those at 48h and 72 h and this seems to be the case in the present study as well. Please use and calculate the 72 h NSE-values rather than the ”highest observed NSE” and present an ROC curve since this may indeed improve this study.

One problem with the 72-h NSE value as the marker of interest is missing data. More than half of the cohort had 2 measurements, but some had missing data. Considering only 72-NSE values would substantially reduce the already small sample size (n=61). We prefer to keep the peak value strategy for the main analysis for the reason you stated at the next point and include your suggestion as a sensitivity analysis in a supplementary file.

3 The 72 hour time point is relevant since no clinician would consider making a decision on withdrawal of active care prior to that time; a decision should rather be postponed to an even later time, as is actually done in the present study! This
protocollized routine is a strength of the present study. The 24h measurement, as a complement to the 72 h sample, is still important in order to see trends; a single blood sample is never enough. Why was not an even later NSE sample analyzed – it would have been relevant since a decision on level of care was routinely postponed until 5 days after cardiac arrest in patients remaining comatose.

Agreed, but we designed the study without later measurement.

4 An improvement would also be to correlate the 72 h samples to best CPC, since other causes than "brain failure" will affect the 3 month outcome. Please add such data.

Best CPC is unclear for us. We did a CPC evaluation at 3 months. The comparison between 72-hour NSE and CPC at 3 months was already reported in the manuscript, page 8 as follows: median 72-h NSE levels: 129.5 (for CPC 4-5, i.e. poor outcome) versus 15.7 (CPC 1-3, i.e. good outcome), p<0.0001.

5 Regarding weaknesses, the issue of hemolysis is addressed in the discussion, but not in the methods section. Were samples analyzed regarding hemolysis? Were samples with visible hemolysis excluded? One cause of hemolysis is the use of an intraaortic ballon pump – was this the case in any of the patients? If so, in which patients? Are those samples "outliers", indicating actual hemolysis?

This point was already discussed by Reviewer Johann Reisinger. Please refer to response 3, Reviewer Johann Reisinger.

Minor essential revisions:

1 Regarding definitions of EEG; would another definition of malignant versus non-malignant have changed its relevance? I am especially interested to know if an isoelectric EEG at 24 h had been considered non-malignant – would this have changed the false positive rate at this time? Consider re-evaluating this definition – we know that some patients recover late and this may be augmented by the use of hypothermia and the use of sedative drugs. I would have expected that a non-malignant EEG would predict a good outcome, as has been shown in recent studies. Is this a result of what definitions were used. Please consider and discuss.

In our paper EEG patterns were classified according to the classification system of Syneck et al. Unfortunately, we recorded EEG results as binary. Therefore we cannot answer your important question.

2 The conclusion that a multimodal approach should be used when a decision on level of care is made is important and relevant to the present study results. The
authors argue that neurophysiological tests (SSEP, EEG) and biochemical markers (NSE) are used as a complement to a clinical neurological examination which is supported by the results in the study.

Thank you for this comment

Figure 1: Please explain how 9+5+7+5 adds up to 24 (!)

Nothing to say other than thank you for seeing this error!
This point is now corrected

Figure 2: Remake and use the 72 h NSE value rather than the "highest observed NSE"

Please refer to response 2 in Major compulsory revisions and examine Additional file now included in the revised version.

Figure 3: This figure is not immediately comprehensible. Please reconsider.

OK, done.