Title

Prognostic Value of Early Phase 1H Magnetic Resonance Spectroscopy and Diffusion Tensor Imaging in Comatose Survivors of Out-of-Hospital Cardiac Arrest - A Sub-Study of the Xe-Hypotheca Trial

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Publication Date

2018-08-20

Peer reviewed

Intensive Care Medicine

Prognostic value of early phase 1H magnetic resonance spectroscopy and diffusion tensor imaging in combination with neuron-specific enolase and motor score in comatose survivors of out-of-hospital cardiac arrest – A Sub-study of the Xe-Hypotheca Trial

--Manuscript Draft--

Manuscript Number:	ICME-D-18-01849R1
Full Title:	Prognostic value of early phase 1H magnetic resonance spectroscopy and diffusion tensor imaging in combination with neuron-specific enolase and motor score in comatose survivors of out-of-hospital cardiac arrest – A Sub-study of the Xe-Hypotheca Trial
Article Type:	Original
Funding Information:	Academy of Finland (FI) (-) — Dr Risto O. Roine State Research Funding (-) — Dr Timo Laitio
Abstract:	Purpose Guidelines recommend brain imaging for neurological prognostication after an out-of-hospital cardiac arrest (OHCA). We aimed to evaluate the predictive accuracy of diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy (1H-MRS) combined with selected clinical examinations and neuron-specific enolase (NSE) for poor neurological outcome after OHCA. Methods This study was a two-centre randomised phase 2 clinical drug trial. 110 comatose OHCA patients were randomised to receive either therapeutic hypothermia treatment alone or inhaled xenon in combination with hypothermia, each for 24 hours. The predictive accuracy of DTI, 1H-MRS, motor score, NSE and standard pupillary light reflex for poor neurological outcome (mRS 3−6) at six months were assessed by area under the receiver operating characteristic (ROC) curve. Results Of the randomised patients 92 had a complete set of DTI, 1H-MRS and mRS data. The brain imaging was performed in a median (IQR) time of 53 hours (47−64) after OHCA. At six-months, 31 patients had mRS 3−6. The area under ROC curve was 0.73 (95% CI 0.62−0.84) for mean white matter fractional anisotropy, 0.78 (95% CI 0.68−0.88) for ratio of total N-acetylaspartate over total creatine (tNAA/tCr) in basal ganglia, and 0.97 (95% CI 0.94−1.00) for fractional anisotropy and tNAA/tCr combined with NSE and motor score at 72 hours for mRS 3−6. Conclusions The combination of mean fractional anisotropy, tNAA/tCr, NSE and motor score within 72 hours had high prognostic accuracy for poor neurological outcome at six months after OHCA. Current result warrants confirmation in another population of cardiac arrest patients.
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Author Comments:	COVER LETTER Editor-In-Chief Intensive Care Medicine Professor Elie Azoulay We are extremely grateful for the additional time that you granted us to complete and submit our revised manuscript. As the following additions/changes/responses bear out, this time was needed to acquire new data and further searching of the literature to comprehensively address the Editor's and Reviewers' valuable questions, comments, and concerns. We have now addressed each of the concerns raised by the Editor and Reviewers to improve the ability of this study to provide reliable prognostic information in a context-sensitive manner. The main additions include 1.The modified Ranking Score (mRS) is used as the study end-point instead of mortality with scores dichotomized as good (mRS 0-2) and poor (mRS 3-6) functional outcome at six months. Therefore, all results were revised. 2.A more detailed description of our standard prognostication protocol which is also used in routine clinical practice in our intensive care unit to preclude premature decisions to withdraw life-sustaining therapy. The detailed protocol is now included in the revised supplement. 3.Results of additional clinical information (pupillary light reflexes and motor score) and a laboratory test (neuron-specific enolase [NSE]) collected at the time of the imaging study; the combination of these results in combination with the imaging study results are now reported. 4.Results of the diagnostic tests (sensitivity, specificity, PPV and NPV) are presented. It is emphasized that the validity of using the results of these tests for prognostication will need to be tested in another prospective trial including also patients with non-shockable rhythm. 5.Limitations of the study are presented more candidly. After adding these results, we are now able to provide a better appreciation for, and perspective how, the MRI parameters can be used in combination with the commonly used clinical prognostic methods. As a conclusion, the prognostic value of mean fractional anisotropy and tNAA/tCr of 1H-MRS in combination with NSE and motor score at 72 hours was high with an AUC of 0.97 (CI 95% 0.94-1.00) Provoked by your insightful comments and questions, we have responded with a vastly improved product that we hope will now be suitable for publication.

	Sincerely Timo Laitio On behalf of the authors of this submission
Response to Reviewers:	RESPONSE LETTER EDITOR'S Comments COMMENT In particular, some important features of your study limit its applicability as a prognostication study: 1 - no mention of clinical examination is made. Were all included patients comatose and in need of a prognostic study? What was their Glasgow Coma Score (or motor score) at the time of prognostic assessment? RESPONSE: We agree that this study has some features that may limit the broad applicability of the imaging biomarkers in prognosticating outcome. However, we have now addressed each of the concerns raised by the editor and reviewers to improve the ability of this study to provide reliable prognostic information in a context-sensitive manner. Specifically, 1. We have added results of additional clinical information (pupillary light reflexes and motor score) and a laboratory test (neuron-specific enolase [NSE]) collected at the time of the imaging study; the combination of these results in combination with the imaging study results are now reported. The validity of using the results of these tests for prognostication will need to be tested in a prospective trial. 2.All patients were comatose at hospital arrival and during hypothermia treatment. After rewarming was completed regular sedation interruptions were commenced in all patients. To improve the quality of the imaging studies, and in particular limiting movement artifacts, the protocol stipulated that the patient be kept intubated and sedated until the imaging study was performed regardless of neurological status. a.Under "Procedure" in the "Methods" section (Line 148-150) of the revised manuscript we have clarified as follows: "MRI imaging was scheduled to be performed within 16 hours of rewarming i.e. 36-52 hours after OHCA. Patients were kept intubated and sedated (with sedation interruptions after completion of rewarming) until brain imaging was performed regardless of the neurological status." 3.Regarding the motor scores and its timing, we now provide the following elaboration in the Results section (Line 217-221) a. "Nine of the 61 patients with good neurological outcome and 27 out of 31 patients with poor neurological outcome had motor score ≤ 3 at 72 hours after OHCA (Table 2). Ten patients responded appropriately to commands within 48 hours after OHCA; a further 32 patients were responsive to commands between 48 to 72 hours and 25 patients achieved this state later than 72 hours. Twenty-five never achieved a motor score of 6, all of whom all died. " b.Accordingly, Tables 1 and 2 were also revised. 4.During sedation interruption, the motor score and pupillary light reflexes were assessed in all patients. This is now also referred to in the Discussion section (Line 240-253) as follows: a.A single measure motor score of ≤ 3 at 72 hours provided the best prognostic value for poor neurological outcome as compared with other methods; NSE at 72 hours was next best. In earlier studies, a cut-off value ≤ 2 for motor score have revealed low specificity and high sensitivity between 70- 80% [20, 21]. Here we demonstrate similar sensitivity values with higher specificity; the improvement in the latter attribute can be explained by homogenous cardiac arrest population that only included patients with a shockable primary rhythm. Current AUC of 0.85 for NSE is consistent with the values of 0.86 and 0.90 recorded in earlier large studies in TTM-treated patients [22, 23]. However, comparing NSE results among studies may be problematic because cut-off values vary and a consistent threshold limit for 0% false positive ratio has not been recommended [4]. Higher NSE cut-off values predict worse outcome with cut-off values of ≥23 as compared with current cut-off value of 21 [4, 23]. Among clinical tests in this study pupillary light reflex provided the lowest prognostic value although sensitivity and specificity were similar as in two recent large multicenter studies [11, 24]. One explanation may be that in this study the best value of the first 72 hours was used in

order to obtain a value for each patient. As a result, in some patients only assessments of the first 48 hours were applicable, which may have led to higher false positive ratio.
5.In clinical practice imaging studies would not be required if patients have demonstrated their ability to obey commands within 48-72 hours as is also clearly stated in the recent guidelines.
6.In the revised manuscript, the data on NSE, pupillary reactions and motor score within the first 72 hours after OHCA are now included and analyzed together with the MRI parameters as was also requested by other reviewers. After adding these results, we are now able to provide a better appreciation for, and perspective how, the MRI parameters can be used in combination with the commonly used clinical prognostic methods. These results are now presented in revised Table 2 and in revised figure 3 with new ROC curves. Results of the diagnostic tests (sensitivity, specificity, PPV and NPV) are presented in the revised Table 2.
7.Limitations of this study is expressed more candidly, e.g. “First, our results represent a two-center cohort (single country) of a moderate sample size in patients in whom a shockable rhythm was the initial rhythm at time of resuscitation. Therefore, further validation of the results is required in larger number of patients with both shockable and non-shockable primary rhythms.” Please see more details under line-by-line responses (Pages 5-14 in this response letter).

COMMENT 2- while according to the study Methods "clinical outcome was evaluated at six months after OHCA with modified Rankin scale by experienced neurologists", the study outcome was mortality. Please report neurological outcome as well, as suggested by Reviewers (see their comments below)
RESPONSE: We agree and have changed all results according to the requested study endpoint of mRS with scores dichotomized as good (mRS 0-2)and poor (mRS 3-6) functional outcome at six months. Tables 1 and 2, and figures 1-3 are revised accordingly.
COMMENT 3 - were the STARD (Standards for Reporting Diagnostic Accuracy) guidelines used to conduct the study and report its results, according to current standards? For further details, see Bossuyt PM et al, BMJ 2015; 351 doi: https://doi.org/10.1136/bmj.h5527
RESPONSE: We agree and the following information is now added in the revised Methods section (lines 131-132) as follows:
“Study design and methodology was consistent with the STARD guidelines for reporting diagnostic accuracy studies [12].

COMMENT 4 - did you investigate predictive indices other than imaging in that study? You compared your study with the one by Veilly et al. However, in that study the predictive value of MRI variables was interpreted in light of the results of both clinical examination and EEG. Would you provide further clinical predictive variables in order to help putting better your results in context?
RESPONSE: We agree and have included results of NSE, pupillary light reflex and motor score. Regarding this matter, please see our response to the first comment above.

EDITOR'S COMMENTS:

COMMENT:In addition to reviewers' comments, I have a few additional comments:
- a few details concerning the Xe-HYPOTHECA trial should be reported in the main text, especially the most relevant to the aims of the current study (neuroprognostication)
RESPONSE: We have reported the following in the last paragraph of the Introduction (Lines 117-120) as follows:
As defined in the protocol, the purpose of this study was to compare the predictive values for 6-month neurological outcome, dichotomized as good (mRS 0-2) and poor (mRS 3-6), of fractional anisotropy from DTI, with several brain metabolites from 1H-MRS each obtained by MRI along with motor score, NSE and pupillary light reflex performed in comatose survivors within 72 hours after OHCA.

Furthermore, we refer to the trial and its published protocol under ”Participants” in the

revised Methods section (Lines 138-139) as follows:

"We have previously reported the primary and secondary clinical end points of the Xe-HYPOTHECA trial; [8, 13]. The protocol of the Xe-HYPOTHECA trial has also been published [8]."

as well as under "Procedure" in revised Methods (Lines 148-158) as follows:
MRI imaging was scheduled to be performed within 16 hours of rewarming i.e. 36-52 hours after OHCA. Patients were kept intubated and sedated (with sedation interruptions after completion of rewarming) until brain imaging was performed regardless of neurological status. A predetermined prognostication protocol (eAppendix in the Supplement) was used to preclude premature decisions to withdraw life-sustaining therapy. DTI and 1H-MRS results did not inform the outcome prognostication. The clinical outcome was evaluated at six months after OHCA with modified ranking scale (mRS) by experienced neurologists.
After rewarming was completed sedation interruptions were initiated and performed every 6 to 12 hours throughout intensive care stay. Motor score of the Glasgow Coma Scale and standard pupillary light reflex were assessed during each sedation interruption by either trained intensive care nurse or on-duty intensive care physicians. NSE serum concentration (Immuno-Electro-Chemi-Luminescent assay, Roche Diagnostics GmbH, Mannheim, Germany) was determined at hospital arrival, and at 24 hours, 48 hours and 72 hours after OHCA.

COMMENT:- the normality of continuous variables was evaluated visually. Did you perform any specific test to assess data normality?

RESPONSE: We did not use the normality tests (Shapiro-Wilk or Kolmogorov-Smirnov). Experienced statistician (Tero Vahlberg, Department of Biostatistics, Turku University) checked visually the distributions. For normally distributed variables parametric methods were used and for non-normally distributed variables nonparametric methods. Due to positively skewed distribution NSE values were log-transformed for statistical analysis.
COMMENT:- MRI diffusivity (DWI) is only fleetingly mentioned (l. 179) in the Methods and its inclusion in the study is not clear. Please provide further details.
RESPONSE: From the classical diffusion weighted imaging (DWI) data we only refer to the mean diffusivity of basal ganglia. The remainder of the DWI data are always acquired as a "by-product" of the diffusion tensor imaging (DTI) procedure; therefore, there is no separate protocol for acquisition in the manuscript. In the revised Methods section (Lines 160-162) we have clarified as follows:
"DTI and DWI data were acquired using diffusion weighted spin-echo echo planar imaging (SE-EPI) sequence with 20 diffusion encoding directions (see eTable1 in the Supplement for details)."
and again, in the revised Methods section (Line 167)
"and mean diffusivity (MD) of the basal ganglia was assessed from the DWI dataset.."

COMMENT:- line 238: "commenced at 72 hours after ictus". What does "ictus" mean here?
RESPONSE: The paragraph that contains that word ictus (2nd paragraph of the Discussion in the first draft; Lines 237-243) has been omitted in the revised manuscript both to comply with the word limit and because it is redundant as descriptions appear elsewhere as follows:
•revised Introduction section (Line 114-116):
"However, the value of these imaging indices, either alone or in combination with motor score, neuron-specific enolase (NSE) and pupillary light reflex, for predicting poor neurological outcome at early phase after OHCA, has yet to be established."
•revised Methods (Lines 150-152):
"A predetermined prognostication protocol (eAppendix in the Supplement) was used to preclude premature decisions to withdraw life-sustaining therapy. DTI and 1H-MRS results did not inform the outcome prognostication."

COMMENT- lines 288-290: "there is a group of patients with good prognosis despite severe diffusion restriction of the deep grey nuclei". This is appears to be a consequence rather than an explanation of the lack of predictive value of MRI

diffusivity in your study.
RESPONSE: We agree that this sentence is somewhat vague and appreciate the opportunity to clarify. In the study by Velly et al.(11) some survivors had good neurological outcome despite DWI signal changes in the basal ganglia. Conversely, in another study (Ryoo et al, Critical Care Medicine 2015:43:11:2370-2377) the sensitivity and specificity of basal ganglia DWI abnormalities were both low, 54% and 47%, respectively; this is probably due to the fact that some patients with poor neurological outcome had no signal changes in the basal ganglia. Thus, it seems that DWI analysis of the basal ganglia alone is not a reliable marker of poor outcome in these patients, as it can fail both because some patients with poor outcome may have normal basal ganglia in the MRI, and because some patients with good outcome may have quite severe DWI abnormalities. The underlying pathophysiological processes for this phenomenon remain unknown.

As this response is too long to include we have decided to omit the sentence entirely. We have revised the original sentence accordingly.
Lines 271-275 in the revised manuscript:
“In our study, DWI of the basal ganglia alone was a poor predictor of neurological outcome. Previous studies have shown that some patients with poor outcome have no DWI signal changes in the basal ganglia [30], and some patients with even severe diffusion restriction in the basal ganglia may end up with good neurological recovery [11]. This may explain the poor predictive value in our study. The underlying physiological reason for this phenomenon remains unknown.”

Due to the inclusion of additional data the following revisions were made:

Responses line by line:
Topic:
WAS: Prognostic value of early phase 1H magnetic resonance spectroscopy and diffusion tensor imaging in comatose survivors of out-of-hospital cardiac arrest – A Sub-study of a Randomized Clinical Trial (the Xe-Hypotheca Trial)
In the revised manuscript:
NOW: Prognostic value of early phase 1H magnetic resonance spectroscopy and diffusion tensor imaging in combination with neuron-specific enolase and motor score in comatose survivors of out-of-hospital cardiac arrest – A Sub-study of the Xe-Hypotheca Trial
Short Title:
WAS: Prognostication with magnetic resonance imaging after cardiac arrest
In the revised manuscript:
NOW: Prognostication after cardiac arrest
Abstract
Purpose
In the Revised Abstract: Lines 49-50: …“with selected clinical examinations and neuron-specific enolase (NSE) for poor neurological outcome after OHCA.”
And also: Lines 54-55: “The predictive accuracy of DTI, 1H-MRS, motor score, NSE and standard pupillary light reflex for poor neurological outcome (mRS 3-6) at six months”
Abstract Results:
WAS: Of the randomised patients 93 had both DTI and 1H-MRS data available. The brain imaging was performed in a median (inter-quartile range) time of 53 hours (47-64) after OHCA. During the follow-up of six-months 27 patients (28%) died. The area under ROC curve was 0·73 (95% CI 0·61-0·85; P=0·0005) for global white matter fractional anisotropy (FA), 0·76 (95% CI 0·65-0·87; P=0·0001) for ratio of total N-acetylaspartate over total creatine (tNAA/tCr) in basal ganglia, and for a combination of FA and tNAA/tCr 0·84 (0·76-0·93; P < 0·0001) There was no significant difference (P=0·77) in predictive power between FA and 1H MRS.
In the revised Abstract Results:
NOW: Lines 59-63: Of the randomised patients 92 had a complete set of DTI, 1H-MRS and mRS. The brain imaging was performed in a median (IQR) time of 53 hours (47-64) after OHCA. At six-months, 31 patients had mRS 3-6. The area under ROC curve was 0.73 (95% CI 0.62-0.84) for mean white matter fractional anisotropy, 0.78 (95% CI 0.68-0.88) for ratio of total N-acetylaspartate over total creatine (tNAA/tCr) in basal ganglia, and 0.97 for a combination of fractional anisotropy, tNAA/tCr , NSE and motor

score at 72 hours (0.94-1.00).

Abstract Conclusions
WAS: The combined predictive accuracy of global FA and tNAA/tCr of 1H-MRS within 72 hours after OHCA was good for mortality at six months after OHCA and could be used in clinical prognostication in conjunction with other methods.
In the revised Abstract:
NOW: Conclusions: The combination of mean fractional anisotropy, tNAA/tCr, NSE and motor score within 72 hours after OHCA had high prognostic accuracy for poor neurological outcome at six months after OHCA. Current result warrants confirmation in another population of cardiac arrest patients.

Keywords:
WAS: Diffusion tensor imaging, 1H-MRS, Cardiac arrest, Prognostication
NOW: Diffusion tensor imaging, 1H-MRS, Neuro-specific enolase, Motor score, Cardiac arrest, Prognostication

Take home message and Tweet is rewritten accordingly.

Introduction:
Lines 90-91:
WAS: “ranging from 41% to 86%, despite implementation of targeted temperature management and other improvements in the treatment of these patients [1, 2].”
NOW: lines 92-93: “ranging from 41% to 86%, despite implementation of therapeutic hypothermia (also referred to as targeted temperature management) and other improvements in the treatment of these patients [1, 2].”

Line 94:
WAS: “Based on the current guidelines neurological prognostication is usually initiated 72 hours after cardiac arrest [4].”
NOW: “Based on the current guidelines neurological prognostication is recommended in patients who exhibit an extensor motor response to pain at 72 hours or later after cardiac arrest [4].”

Lines 98-99:
WAS: “Therefore, there is an unmet need for new biomarkers to improve the accuracy of early-phase prognostication and the need to identify non-survivors.”
NOW: “Therefore, there is an unmet need for new biomarkers to improve the accuracy of early-phase prognostication in order to identify patients with poor neurological outcome.”

Lines 112-115:
WAS:” … in combination, for predicting mortality at early phase after OHCA, has yet not been established.
As defined in the original study protocol, the purpose of this study was to compare the predictive values for 6-month mortality, of fractional anisotropy from DTI, with several brain metabolites from 1H-MRS each obtained by MRI performed in comatose survivors within 72 hours after OHCA.”
NOW: “…in combination with motor score, neuron-specific enolase (NSE) and pupillary light reflex, for predicting poor neurological outcome at early phase after OHCA, has yet to be established.
As defined in the original study protocol, the purpose of this study was to compare the predictive values for 6-month neurological outcome, dichotomized as good (mRS 0-2) and poor (mRS 3-6), of fractional anisotropy from DTI, with several brain metabolites from 1H-MRS each obtained by MRI along with motor score, NSE and pupillary light reflex performed in comatose survivors within 72 hours after OHCA.”

Methods:
Lines 129-133 in the original manuscript:
WAS: “Consecutive comatose survivors of out-of-hospital cardiac arrest admitted to the Turku and Helsinki University hospitals between August 2009 and September 2014 were screened for eligibility. The main criteria for inclusion were witnessed cardiac arrest from an initial shockable rhythm (i.e., ventricular fibrillation or non-perfusing ventricular tachycardia) and return of spontaneous circulation within 45 minutes.

	Detailed inclusion and exclusion criteria are listed in eAppendix in the Supplement." NOW: Lines 135-137: "Consecutive comatose survivors of witnessed out-of-hospital cardiac arrest from an initial shockable rhythm admitted to the Turku and Helsinki University hospitals between August 2009 and September 2014 were screened for eligibility. Detailed inclusion and exclusion criteria are listed in eAppendix in the Supplement." Lines 134-137: WAS: "We have previously reported the primary and secondary clinical end points of the Xe-HYPOTHECA trial; xenon combined with hypothermia confers both neuroprotection, by attenuating the brain white matter injury, as well as cardioprotection by attenuating myocardial damage more than hypothermia alone in comatose survivors of cardiac arrest [8,12]. The protocol of the Xe-HYPOTHECA trial has also been published [8]." NOW: Lines 138-139: "We have previously reported the primary and secondary clinical end points of the Xe-HYPOTHECA trial [8, 12]. The protocol of the Xe-HYPOTHECA trial has also been published [8]." Lines 140-146 in the original manuscript: WAS: "The patients were allocated in a 1:1 ratio with random block sizes of 4, 6, and 8 to receive either therapeutic hypothermia treatment alone for 24 hours or inhaled xenon (LENOXe, Air Liquide Medical GmbH, Düsseldorf, Germany) in combination with hypothermia for 24 hours [8]. Personnel involved in the treatment of the patient could not be blinded due to practical and safety considerations; however, the magnetic resonance imaging analysis of white matter injury was operator-independent, and the neurological end-point evaluators as well as the patients were blinded to the treatment. Neither DTI nor 1H-MRS data were made available to the clinicians treating the patients to avoid the "self-fulfilling prophecy" effect." NOW: lines 142-145: "The patients were allocated in a 1:1 ratio with random block sizes of 4, 6, and 8 to receive either therapeutic hypothermia treatment alone for 24 hours or inhaled xenon (LENOXe, Air Liquide Medical GmbH, Düsseldorf, Germany) in combination with hypothermia for 24 hours as described earlier [8]. The neurological end-point evaluators as well as the patients were blinded to the treatment. " Lines 148-150: WAS: "MRI imaging was scheduled to be performed within 16 hours of rewarming i.e. 36-52 hours after OHCA. The clinical outcome was evaluated at six months after OHCA with modified ranking scale (mRS) by experienced neurologists." NOW: "MRI imaging was scheduled to be performed within 16 hours of rewarming i.e. 36-52 hours after OHCA. Patients were kept intubated and sedated (with sedation interruptions after completion of rewarming) until brain imaging was performed regardless of the neurological status. A predetermined prognostication protocol (eAppendix in the Supplement) was used to preclude premature decisions to withdraw life-sustaining therapy. DTI and 1H-MRS results did not inform the outcome prognostication. The clinical outcome was evaluated at six months after OHCA with modified ranking scale (mRS) by experienced neurologists. After rewarming was completed sedation interruptions were initiated and performed every 6 to 12 hours throughout intensive care stay. Motor score of the Glasgow Coma Scale and standard pupillary light reflex were assessed during each sedation interruption by either trained intensive care nurse or on-duty intensive care physicians. NSE serum concentration (Immuno-Electro-Chemi-Luminescent assay, Roche Diagnostics GmbH, Mannheim, Germany) was determined at hospital arrival, and at 24 hours, 48 hours and 72 hours after OHCA. " EEG was recorded only if it was clinically indicated. Line 155: WAS: FSL software library (version 5.0, Analysis Group, FMRIB, Oxford, United Kingdom) was used for processing Line 163: NOW: FSL software library (version 6.0, Analysis Group, FMRIB, Oxford, United Kingdom) was used for processing Lines 158-159:

WAS: “Global fractional anisotropy value of white matter was calculated as a mean value of all the voxels in the skeleton (see eMethods in Supplement for details).”
NOW: Lines 169-171: “Mean fractional anisotropy value of white matter was calculated as a mean value of all the voxels in the skeleton, and mean diffusivity (MD) of the basal ganglia was assessed from the DWI dataset (see eMethods in Supplement for details).”

Statistical Analysis:
Added information in the revised section of statistical analysis:
Terms “Survivor and non-survivor” is now changed to “groups of mRS 0-2 and mRS 3-6” or to “groups”.
Lines 185-186: “…and NSE at 48 and 72 hours after OHCA..”
Lines 187-188: “NSE values were log-transformed for statistical analysis due to positively skewed distribution.”
Lines 199-201: “Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for each prognostic variable were calculated. Optimal cut-off values were chosen by using Youden Index (sensitivity+specificity-1).”
Results:
Lines 198-207:
WAS: “Of the 224 patients screened for eligibility for the Xe-HYPOTHECA trial, 110 were included. Of the 224 patients screened for eligibility for the Xe-HYPOTHECA trial, 110 were included. Of these, 97 completed the neuroimaging protocol in a median (inter-quartile range) time of 53 hours (47-64) after OHCA. Both 1H-MRS and DTI data were available for 93 patients (Fig. 1).
Patients who died during the follow-up of six months were older (P=.002), had higher frequencies of diabetes (P = .048) and status epilepticus (P<.0001), and longer time to ROSC (P=.0004). In addition, selected laboratory values of pH (P=.002), and creatine (P=.001) from ICU admission to target temperature, and cumulative dose of norepinephrine during the first 72 hours (P=.002) were significantly higher in the non-survivors than in the survivors (Table 1). During the follow-up to six months, there were 27 deaths (28%). According to mRS at six months after OHCA, 61 patients had good (mRS 0-2) and 31 patients had poor (mRS 3-6) neurological outcome (eTable 3). One patient was withdrawn six days after the index event by the next of kin.”
NOW: Lines 207-212: “Of the 224 patients screened for eligibility for the Xe-HYPOTHECA trial, 110 were included. Of these, 97 underwent magnetic resonance imaging in a median (inter-quartile range) time of 53 hours (47-64) after OHCA and 93 had 1H-MRS, DTI and DWI data available (Fig. 1).
One patient was withdrawn six days after the index event by the next of kin and therefore 92 out of 93 had applicable mRS data (Fig. 1). At six months after OHCA, 61 patients had good (mRS 0-2) and 31 patients had poor (mRS 3-6) neurological outcome. Patient demographics and clinical characteristics are presented in table 1. ”

pH and diabetes did not differ between the groups of good and poor neurological outcome.

Lines 217-224: New information:
“Pupillary light reflex, NSE and motor score
Pupillary light reflex within 72 hours did not differ. NSE at 48 and 72 hours after OHCA were significantly higher in patients with poor neurological outcome than in the patients with good neurological outcome at six months (Table 2). 9 of the 61 patients with good neurological outcome and 27 out of 31 patients with poor neurological outcome had motor score ≤ 3 at 72 hours after OHCA (Table 2). Ten patients responded appropriately to commands within 48 hours after OHCA; a further 32 patients were responsive to commands between 48 to 72 hours and 25 patients achieved this state later than 72 hours. 25 never achieved a motor score of 6, all of whom all died.”

Lines 209-214:
WAS:
“DTI and 1H-MRS results
Global fractional anisotropy values of the DTI, and tNAA/tCr and tNAA/tCho ratios of the 1H-MRS were significantly higher in the survivors and in patients with good neurological outcome than in the non-survivors and in patients with poor neurological outcome. Other DTI and 1H-MRS measures did not differ between the groups (Table 2 and eTable 4). Results of the tract-based spatial statistics analysis are visualized with a

statistical parametric map (Fig. 2)."

NOW:
"DTI, DWI and 1H-MRS results
Global fractional anisotropy values of the DTI, and tNAA/tCr and tNAA/tCho ratios of the 1H-MRS were significantly higher in patients with mRS 0-2 than in in patients with mRS 3-6 (Table 2). Results of the tract-based spatial statistics analysis are visualized with a statistical parametric map (Fig. 2). Results of the age-, sex-, treatment-, and site-adjusted survival analyses are presented in eFigure 2 in the supplement.

Lines 216-228 rewritten:
WAS:
"Survival analysis
The age-, sex-, treatment-, and site-adjusted hazard ratio (HR) for death for 0.01-unit increase in the global fractional anisotropy was 0.81 (95% CI 0.70-0.95; P=.009). The adjusted HRs for 0.1-unit increase in tNAA/tCr, in tNAA/tCho, in tCho/tCr and for 10-unit increase in mean diffusivity of the basal ganglia were 0.69 (95% CI 0.55-0.86; P=.001), 0.95 (95% CI 0.90-0.99; P=.021), 0.99 (95% CI 0.89-1.10; P=.88) and 0.97 (95% CI 0.92-1.03; P=.37), respectively (eFig. 2)."
NOW: The following sentence is now under the revised section of DTI, DWI and 1H-MRS results: "Results of the age-, sex-, treatment-, and site-adjusted survival analyses are presented in eFigure 2 in the supplement."

Lines 223-228:
WAS:
"Receiver operating characteristic analysis
Area under the curve for predicting death at six months was 0.73 (95% CI 0.61-0.85; P=.0005) for fractional anisotropy, 0.76 (95% CI 0.65-0.87; P=.0001) for tNAA/tCr, and 0.84 (0.76-0.93; P<.0001) for a combination of fractional anisotropy and tNAA/tCr (Fig. 3), indicating fair predictive value for both fractional anisotropy and tNAA/tCr alone, and good predictive value for the combination. There was no significant difference (P = .77) in predictive power between fractional anisotropy and tNAA/tCr."

NOW: lines 229-234: "Receiver operating characteristic analysis and diagnostic tests
Both motor score and NSE at 72 hours had better diagnostic values than mean diffusivity, fractional anisotropy or 1H-MRS measures for poor neurological outcome (Table 2, Fig 3). There was no significant difference (P=0.53) in predictive power between fractional anisotropy and tNAA/tCr. Area under the curve was 0.92 (95 % CI 0.86 – 0.97) for a combination of fractional anisotropy, NAA/Cr and NSE and 0.97 (95% CI 0.94 – 1.00) when FA and tNAA/tCr were combined with NSE and motor score at 72 hours after OHCA (Fig 3). "

Discussion:
First paragraph, Lines 232-236: is rewritten
WAS: "The main finding of this study was that predictive power of global mean fractional anisotropy of white matter in combination with tNAA/tCr of the basal ganglia was better than either one alone and therefore the combination had additive prognostic value at the early subacute phase within 72 hours for mortality at six months after OHCA. In this study, quantitative analysis of the mean diffusivity of the basal ganglia did not provide any independent prognostic value."
NOW: "The main finding of this study was that mean fractional anisotropy of white matter and tNAA/tCr of the basal ganglia in combination with NSE and motor score at 72 hours had high prognostic value for poor neurological outcome at six months after OHCA. Quantitative analysis of the mean diffusivity of the basal ganglia or standard pupillary light reflex did not provide any independent prognostic value."

Second paragraph, lines 237-243 in the original manuscript, is omitted.

Second paragraph in the revised manuscript (NEW):
"A single measure motor score of ≤ 3 at 72 hours provided the best prognostic value for poor neurological outcome as compared with other methods; NSE at 72 hours was next best. In earlier studies, a cut-off value ≤ 2 for motor score have revealed low specificity and high sensitivity between 70- 80% [18, 19]. Here we demonstrate similar

sensitivity values with higher specificity; improvement in the latter attribute can be explained by homogenous cardiac arrest population that only included patients with a shockable primary rhythm. Current AUC of 0.85 for NSE is consistent with the values of 0.86 and 0.90 recorded in earlier large studies in TTM-treated patients [20, 21]. However, comparing NSE results among studies may be problematic because cut-off values vary and a consistent threshold limit for 0% false positive ratio has not been recommended [4]. Higher NSE cut-off values predict worse outcome with Youden cut-off values of ≥23 as compared with current Youden cut-off value of 21 [4, 21]. Among clinical tests in this study pupillary light reflex provided the lowest prognostic value although sensitivity and specificity were similar as in two recent large multicenter studies [11, 22]. One explanation may be that in this study the best value of the first 72 hours was used in order to obtain a value for each patient. As a result, in some patients only assessments of the first 48 hours were applicable which may have led to higher false positive ratio.“

Paragraph 3, lines 247-250 in the original manuscript, is omitted: “Our earlier report on the same population demonstrated that significantly lower fractional anisotropy in non-survivors than in the survivors was mainly attributed to an increased radial diffusivity indicating demyelination in the early phase after cardiac arrest [8].
REASON: is already described in the introduction

Paragraph 4, lines 251-255 in the original manuscript, omitted: “While behavior of fractional anisotropy values can be diverse in the hyperacute ischaemic phase, they are almost always decreased thereafter in the subacute and in the chronic stages [22]. For these reasons, it is important to note that in the current study all patients were scanned within a narrow time window of a median of 53 hours and less than 72 hours after cardiac arrest. Furthermore, identical imaging protocols and MRI devices were used in the two participating centres.”
REASON: 1) mostly described in the methods, 2) to comply with word limit.

Paragraph 6; Lines 263-270 in the original manuscript is rewritten:

WAS: “It is well documented that the white matter injury evolves over a span of days to weeks during the ongoing ischemic process after cardiac arrest [25]. This may result in distinctive predictive value of white matter fractional anisotropy for specific imaging time windows after cardiac arrest. This contention is supported by our results demonstrating lower predictive value of global fractional anisotropy with an area under ROC of 0.73 obtained within 3 days after OHCA as compared to later time windows between 7 and 28 days in the study by Velly and colleagues [11]. On the other hand, the lower area under ROC observed in this study could be explained at least partly by differences in populations as was demonstrated by the proportion of non-survivors of 29 % in this study and 78 % in the study by Velly and colleagues [11].”

NOW:End of paragraph 3, lines 273-275: “Current lower prognostic value for fractional anisotropy at early subacute phase can be partly due to evolving white matter injury over time leading to distinctive predictive value for specific imaging time windows [27].”
REASON: word limit

Lines 271-278 is rewritten:
WAS:“NAA is ubiquitous in the central nervous system, and is a marker of mature neurons and their integrity; persistent reductions in NAA have been used as a marker of neuronal loss and extent of neurological damage [9,11,26]. In addition, earlier studies in patients with stroke and cardiac arrest have demonstrated that a reduced level of NAA have prognostic value for clinical outcome [9,11,26],This was further supported by the present study revealing that ratios of tNAA/tCr and tNAA/tCho in the basal ganglia were significantly lower in non-survivors than in survivors and were independent predictors for mortality at six months. However, the predictive accuracy of the ratio of tNAA/tCr at early subacute stage was somewhat lower than the accuracy observed during 7 and 28 days after cardiac arrest [11].”

NOW:Lines 276-280 in the revised discussion: “NAA is ubiquitous in the central nervous system, and is a marker of the integrity of mature neurons; persistent reductions in NAA have been used as a marker of neuronal loss and extent of neurological damage and have prognostic value for outcome in stroke and cardiac

arrest [9, 11, 28]. These earlier interpretations were supported by the present study revealing that ratios of tNAA/tCr and tNAA/tCho in the basal ganglia are independent predictors for poor neurological outcome at six months. “

Lines 279-284 is rewritten:
WAS: While our study demonstrates good prognostic value for the combination of tNAA/tCr and global fractional anisotropy with an area under ROC of 0.84, the predictive accuracy of these neuroimaging markers may be insufficient to be used in isolation for prognosticating outcome in OHCA patients during the first 72 hours after ictus. However, inclusion of tNAA/tCr and global fractional anisotropy levels in possible future quantitative prognostic models could be considered, as they provide additional information to clinical, neurophysiologic, and biochemical variables.

NOW: In this study, a combined model of fractional anisotropy, tNAA/tCr, NSE and motor score provided high prognostic value with AUC of 0.97 as early as 72 hours for all consecutively enrolled OCHA patients with ventricular fibrillation as a primary rhythm. As a strength of the current new model fractional anisotropy, tNAA/tCr and NSE are observer independent methods and provided high accuracy even without motor score. Although motor score, NSE and combination of fractional anisotropy and tNAA/tCr provided good prognostic values, none could be used as a singular method due to insufficient accuracy. These results warrants further confirmation in another population including also patients with asystole and PEA as primary rhythm.
REASON: added new data and results

Lines 285-290 in the original manuscript:
WAS: “In this study, quantitative analysis of the mean diffusivity of the basal ganglia did not provide any independent prognostic value. This was clearly demonstrated by almost identical mean diffusivity of the deep grey matter nuclei between survivors and non-survivors. While some previous studies have demonstrated predictive value for qualitative analysis of diffusion changes in the basal ganglia, they have also shown that there is a group of patients with good prognosis despite severe diffusion restriction of the deep grey nuclei [11,27]. This phenomenon may explain the lack of predictive value of mean diffusivity in our study.”
NOW: In our study, DWI of the basal ganglia alone was a poor predictor of neurological outcome. Previous studies have shown that some patients with poor outcome have no DWI signal changes in the basal ganglia [30], and some patients with even severe diffusion restriction in the basal ganglia may end up with good neurological recovery [11]. This may explain the poor predictive value in our study. The underlying physiological reason this phenomenon remains unknown.

Limitations:
Removed text; lines 293-295 in the original manuscript: “in a sub-study of the Phase 3 XePOHCAS clinical trial (NCT03176186), diffusion tensor imaging will be performed in a similar manner to that undertaken in the current trial.”
WAS (lines 292-293): “First, our results represent a two-center cohort of patients in a single country with only a moderate sample size. Therefore, further validation of the results is required”
NOW: “First, our results represent a two-center cohort (single country) of a moderate sample size in patients in whom a shockable rhythm was the initial rhythm at time of resuscitation. Therefore, further validation of the results is required in larger number of patients with both shockable and non-shockable primary rhythms.”

Added limitations in the revised manuscript:
“Third, due to a possible bias caused by the neuroprotective effect of the xenon on the prognostic value of the current metrics all statistical analyses were adjusted with the treatment group. Fourth, our model was limited by small number of patients with study end point and therefore all variables with predictive value for poor outcome could not be included.”

Revised Figure legends:
Figure 1.:
Flow diagram
Flow of the participants in the study Prognostic value of early phase 1H magnetic

	resonance spectroscopy and diffusion tensor imaging in combination with neuron-specific enolase and motor score in comatose survivors of out-of-hospital cardiac arrest – A Sub-study of the Xe-Hypotheca Trial. Figure 2: WAS: Whole brain visualization of the results of cerebral white matter damage White matter damage leads to a loss of microstructural organization that can be quantified by the loss of directionality in the diffusion of water molecules in the white matter tracts. Fractional anisotropy is a scalar value representing this directionality of water diffusion; lower fractional anisotropy values are indicative of less organized diffusion and are an index of more extensive white matter damage. The visualization presents the results of the voxel-wise tract-based spatial statistics analysis of fractional anisotropy values between survivors and non-survivors. Voxels with significantly ($P < 0.05$, family-wise error corrected for multiple comparisons) lower fractional anisotropy values in non-survivors were identified and are shown in red in the statistical visualization (i.e., 54.1% of all 123994 analyzed voxels), whereas the areas in which there was no significant difference in fractional anisotropy values between the groups are shown in green (i.e., 45.9%of all analyzed voxels). According to the Johns Hopkins University white matter tractography atlas [17], the tract-wise distribution of the voxels (percentages in parentheses below) with significantly ($P < .05$; family-wise error corrected for multiple comparisons) lower fractional anisotropy in non-survivors (marked red in the figure) were as follows: cingulum (cingulate gyrus) (40.5%), cingulum (hippocampal region) (78.8%), forceps minor (70.7%) and major (43.2%), superior longitudinal fasciculus (51.5%), inferior longitudinal fasciculus (50.2%), anterior thalamic radiation (62.2%), inferior fronto-occipital fasciculus (54.2%), corticospinal tract (50.8%), uncinate fasciculus (69.1%), and the body of corpus callosum (83.9%). NOW: White matter damage leads to a loss of microstructural organization that can be quantified by the loss of directionality in the diffusion of water molecules in the white matter tracts. Fractional anisotropy is a scalar value representing this directionality of water diffusion; lower fractional anisotropy values are indicative of less organized diffusion and are an index of more extensive white matter damage. The visualization presents the results of the voxel-wise tract-based spatial statistics analysis of fractional anisotropy values between patients with good (mRS 0-2) and poor (mRS 3-6) 6-month neurological outcome. Voxels with significantly ($P < 0.05$, family-wise error corrected for multiple comparisons) lower fractional anisotropy values in patients with poor neurological outcome were identified and are shown in red in the statistical visualization (i.e., 78.2% of all 123994 analysed voxels), whereas the areas in which there was no significant difference in fractional anisotropy values between the groups are shown in green (i.e., 21.8% of all analysed voxels). According to the Johns Hopkins University white matter tractography atlas [18], the tract-wise distribution of the voxels (percentages in parentheses below) with significantly ($P<0.05$; family-wise error corrected for multiple comparisons) lower fractional anisotropy in non-survivors (marked red in the figure) were as follows: cingulum (cingulate gyrus) (88.0%), cingulum (hippocampal region) (81.4%), forceps minor (92.0%) and major (71.7%), superior longitudinal fasciculus (85.3%), inferior longitudinal fasciculus (80.5%), anterior thalamic radiation (85.1%), inferior fronto-occipital fasciculus (84.7%), corticospinal tract (79.9%), uncinate fasciculus (89.3%), and the body of corpus callosum (98.0%). Figure 3: WAS: Receiver operating characteristic curves of fractional anisotropy, tNAA/tCr and the combined model. NOW: Receiver operating characteristic curves of fractional anisotropy combined with tNAA/tCr, fractional anisotropy combined with NAA/tCr and NSE at 72 hours, and the combination of fractional anisotropy, tNAA/tCr, NSE and motor score at 72 hours. Table 1: Revised according to the new results Table 2: Revised according to the new results Figure 1: revised : added information: one patient not having mRS due to withdrawal by the next of kin Figure 2: TBSS of fractional anisotropy is reanalyzed due to new study endpoint. Please see also the revised figure legend. Figure 3: revised according to revised combinations

References in the revised manuscript:
References 18, 19, 22, and 27 have been removed
References 12, 19, 20, 21, 22, 23, 24 have been added
Number of authors up to 10 is now included in the revised references.

REVIEWERS' COMMENTS:

Reviewer #1:
COMMENT: I have read with great attention and interest the results of the Xe-Hypotheca trial group about MRI outcome prediction.
Major comments:
1. As recognised by the authors, the sample size is quite small when compared to recent studies in the field (e.g. Oddo M et al. ICM Dec 2018; n=456 pts)
RESPONSE: We agree that Xe-Hypotheca has some flaws (e.g. the relatively small sample size) that limit its generalized applicability for prognostication. In the revision we have addressed concerns raised by the editor and reviewers to improve its applicability to provide reliable prognostication information in a context-sensitive manner. Thus motivated, we have added results of NSE, pupillary light reflexes, and motor score, and discussed these new results accordingly. It is also clear that current results are not definitive and must be confirmed prospectively in another population of cardiac arrest patients (also mentioned in the “Conclusions” and “Limitations” sections).
After adding data of pupillary reactions, NSE and motor score we have now discussed current results in the light of the results revealed by Oddo et al. in the revised Discussion (Lines 240-253) as follows:
“A single measure motor score of ≤ 3 at 72 hours provided the best prognostic value for poor neurological outcome as compared with other methods; NSE at 72 hours was the next best. In earlier studies, a cut-off value ≤ 2 for motor score have revealed low specificity and high sensitivity between 70- 80% [20, 21]. Here we demonstrate similar sensitivity values with higher specificity; improvement in the latter attribute can be explained by homogenous cardiac arrest population that only included patients with a shockable primary rhythm. Current AUC of 0.85 for NSE is consistent with the values of 0.86 and 0.90 recorded in earlier large studies in TTM-treated patients [22, 23]. However, comparing NSE results among studies may be problematic because cut-off values vary and a consistent threshold limit for 0% false positive ratio has not been recommended [4]. Higher NSE cut-off values predict worse outcome with cut-off values of ≥23 as compared with current cut-off value of 21 [4, 23]. Among clinical tests in this study pupillary light reflex provided the lowest prognostic value although sensitivity and specificity were similar as in two recent large multicenter studies [11, 24]. One explanation may be that in this study the best value of the first 72 hours was used in order to obtain a value for each patient. As a result, in some patients only assessments of the first 48 hours were applicable which may have led to higher false positive ratio.”

In addition in the revised Limitations there are further caveats/qualifications as follows: (Lines 280-283)
”First, our results represent a two-center cohort (single country) of a moderate sample size in patients in whom a shockable rhythm was the initial rhythm at time of resuscitation. Therefore, further validation of the results is required in larger number of patients with both shockable and non-shockable primary rhythms.”
Lines 289-291:
“Fourth, our model was limited by small number of patients with study end point and therefore all variables with predictive value for poor outcome could not be included.”

COMMENT 2. Also, the studied cohort includes a selected population of CA patients with a very low mortality rate (28%) at 6 months
RESPONSE: Per study protocol only OHCA patients with ventricular fibrillation as a primary rhythm were considered eligible. In this study, mortality rate at 6-months was 34.5% in the control group and 27.5% in the xenon group (Laitio R. et al JAMA 2016;315(11):1120-1128). Mortality results from Xe-Hypotheca were consistent with a recent large prospective observational study in Finnish population (FINNRESUSCI study) revealing a mortality rate of 38.2% at 1-year after OHCA in patients with ventricular fibrillation as a primary rhythm.

COMMENT 3. Given this was a neuro-prognostication study, it is unclear why the outcome endpoint selected was mortality rather than favorable neurological recovery, based on mRS dichotomization
RESPONSE: This is an important point and hence we have now used mRS dichotomization as an outcome endpoint in the revised manuscript. Accordingly all results in the tables and figures have been revised.

COMMENT 4. The results of the ROC curve analysis shows good predictive power for mortality, but actually this predictive power does not tell us the exact prognostic value in clinical practice.
Please see our response to comment #5 below.

COMMENT 5. Concerning my previous comment, and in order to provide more useful data with regard to clinical practice and utility for the clinicians, the authors need to report Specificity, Sensitivity, PPV, NPV, TP, TN, and FPR for each prognostic variable selected. Results are too descriptive in nature: so far, base don the data we have, we can say that the severity of lesions seen on MRI is associated with mortality. But we still do not know how to use these data in clinical practice.
RESPONSE: We agree and have now added results of the diagnostic tests as was requested by the Reviewer. These results are now presented in the revised Table 2. In addition, a combination of the best MRI parameters and clinical examinations were analysed and added to the revised manuscript (i.e. revised Table 2 and revised Figure 3) and discussed accordingly and as described above in the response to the first comment. In addition, in the revised Discussion (Lines 272-278) the following has been added:
“In this study, a combined model of fractional anisotropy, tNAA/tCr, NSE and motor score provided high prognostic value with AUC of 0.97 as early as 72 hours for all consecutively enrolled OCHA patients with ventricular fibrillation as a primary rhythm. As a strength of the current new model fractional anisotropy, tNAA/tCr and NSE are observer independent methods and provided high accuracy even without motor score. Although motor score, NSE and combination of fractional anisotropy and tNAA/tCr provided good prognostic values, none could be used as a singular method due to insufficient accuracy. These results warrants further confirmation in another population including also patients with asystole and PEA as primary rhythm.”

COMMENT 6. What was the proportion of survivors and non-survivors that were accurately predicted thanks to the MRI data ?
RESPONSE: Please see our response to the first comment by Reviewer #1. The MRI parameters, alone, are inadequate to prognosticate effectively; rather, the combination of FA, tNAA/tCr, NSE and motors score provides an AUC of 0.97 (95% CI 0.94-1.00). As requested we have now used mRS dichotomization as an outcome endpoint in the revised manuscript. Accordingly all results in the tables and figures have been revised. Chosen cut-off point of each parameter was based on Youden Index. Hopefully, the results of the diagnostic tests, now presented in the revised Table 2, provide information to address this comment. In addition, a paragraph has been amended in the revised Discussion (Lines 272-278; see above).

COMMENT 7 What is the value of MRI when combined with other tools, in particular with pupillary reactivity and with tomato-sensory evoked potentials?
RESPONSE: We have now included results of NSE, pupillary reactions and motor score. Unfortunately, somato-sensory evoked potentials were not used. The diagnostic value of each parameter is now analysed separately and the best MRI parameters have been analysed in combination with the best clinical examinations. The results are presented in the revised Table 2 and discussed as described above. The AUC of the combination of FA, tNAA/tCr, motor score, NSE is 0.97 and without motor score AUC is 0.92.

COMMENT 8. The results of this study are not in agreement with a previous study by Velly et al. (Lancet Neurology 2018). Although the authors already discuss this, I think this point must be better integrated in the discussion. The clinical implication of the present study is that quantitative MRI is insufficient to diagnose outcome with accuracy. Please reconsider this point and put these results into a better perspective.
RESPONSE: Please also see our responses above. We have added results of NSE,

motor score and pupillary reflexes, and analysed the diagnostic value with sensitivity, specificity, NPV and PPV alone and in combination with the best MRI parameters (i.e. FA and tNAA/tCr). We have added the discussions as described above in responses to the comments #1, 5 and 6 above and have examined the results in an appropriate clinical perspective. Please see also our line-by-line descriptions of the changes above.

Reviewer #2: This is a well written paper re-enforcing known results and adding new information: 1. FA metrics are less sensitive at early stage of cardiac arrest than after 7 days, 2. combining DTI and spectometry has more prognostic value than using each of the metrics alone.

COMMENT: DTI metrics highly depend on acquisition parameters on one hand and on potential artefacts on the other hand. A strict protocol of quality check analysis must be followed when assessing these metrics. Please describe this quality check pipeline together with the deviations observed. Describe the cut-off chosen to discard a potential exam. Specifically explain why 4 exam were not taken into account.
RESPONSE: Four exams were not included because these patients did not have 1H-MRS data although they had DTI data. This matter is also presented in the figure 1. Youden Index was used for all cut-off points as is also described in the revised section of statistical analysis.
Since patients were kept intubated and sedated during MRI scan, we expected to obtain very high quality DTI data without motion artefacts. However, visual check of the data and automatic QC using DTIprep were still performed. In fact, there was severe motion detected in case of three patients. Our DTI protocol was such that there was first one image without diffusion encoding (b=0 s/mm2) and then diffusion weighted images with 20 different diffusion encoding directions (b=1000 s/mm2). This data was acquired twice (averages/nex=2). Because of this, we had still data from all 20 diffusion encoding directions. However, there was one subject with more severe motion and DTIfit had to be performed by using only 16 independent diffusion encoding directions.

Further information about automatic quality check with DTIprep is now added to supplement. DTIprep performed following steps:
Diffusion information checks (ensuring correct diffusion gradient orientations, gradient b-values). Following parameters were used for this step:
bValueAcceptaplePercentageTolerance 0.0050,
GradientToleranceForSameness_degree 1.00

Inter-slice brightness artifact detection via normalized correlation analysis between successive slices within a single DWI volume. Following parameters were used for this step:

Check Times: 0, HeadSkipSlicePercentage 0.1, TailSkipSlicePercentage: 0.1, correlationDeviationThresholdBaseline: 3 correlationDeviationThresholdGradient: 3.5

Interlaced correlation analysis for detection and removal of “venetian blind” artifacts and motion within a single DWI volume. Following parameters were used for this step.

CorrelationThresholdBaseline: 0.8182, CorrelationThresholdGradient: 0.7588, CorrelationStedvTimesBaseline: 2.5, CorrelationStdevTimesGradient: 3, TranslationThreshold: 2.3, RotationThreshold: 0.5

Co-registration to an iterative average over all the baseline images. Following parameters were used for this step.
Stop threshold: 0.02

Eddy-current and motion artifact correction, including appropriate gradient direction adjustments. Following parameters were used for this step.

EDDYMOTION_: NumberOfIterations: 1000, NumberOfSamples: 100000, TranslationScale: 1000, MaxStepLength: 0.200, MinStepLength: 0.00010, RelaxFactor: 0.5
GRADIENT_: TranslationThreshold: 2.3021, RotationThreshold: 0.5
In the revised supplement, page 3: Following information is added:

	“Following steps were performed. Diffusion information checks (ensuring correct diffusion gradient orientations, gradient b-values). Inter-slice brightness artifact detection via normalized correlation analysis between successive slices within a single DWI volume. Interlaced correlation analysis for detection and removal of “venetian blind” artifacts and motion within a single DWI volume. Co-registration to an iterative average over all the baseline images. Eddy-current and motion artifact correction, including appropriate gradient direction adjustments. Residual motion detection to ensure all DWI volumes are well registered.” COMMENT: The effect of Xenon on FA metrics should also be emphasized. The potential bias introduced by the experimental protocol on the prognostic value of DTI metrics should be specifically discussed: please add a chapter to the discussion accordingly. RESPONSE: We agree that the xenon treatment may have introduced a potential bias on the prognostic value of the MRI metrics, especially since our previous study demonstrated that Xenon treatment confers a neuroprotective effect in comatose survivors of cardiac arrest. Because of this possible confounder, the treatment group was used as a covariate in all statistical analyses in this study. We have added a chapter in the revised Discussion (Line 287-289) as follows: “Third, due to a possible bias caused by the neuroprotective effect of the xenon on the prognostic value of the current metrics all statistical analyses were adjusted with the treatment group.” COMMENT: Line 158: the authors did not actually measured the global WM FA but instead the average FA of the WM contained in the TBSS space. Correct accordingly. RESPONSE: We agree. In the original manuscript we used the term ”global FA” to emphasize the fact that no specific predetermined region of interest was used for the FA measurements. We agree that this term is somewhat misleading and have corrected accordingly. “Mean” is now used instead of “Global” throughout the manuscript Reviewer #3: Koskensalo and colleagues have conducted a post hoc analysis of a patient material included in a pilot trial on the use of Xenon during post cardiac arrest care. The current study must be seen as hypothesis generating only as the patient material, design does not allow for a proper prognostic study. In addition this study deal with a very selected group of patients treated at two institutions only. RESPONSE: We agree that this study has some features that limit its generalizability as a prognostication study. However, we have now tried to address all the concerns raised by the editor and reviewers to improve the ability of this study to provide reliable prognostic information in the clinical context. Therefore, we have added results of NSE, pupillary light reflexes and motor score and discussed the results accordingly. It is also clear that current results must be prospectively confirmed in another population of cardiac arrest patients (also mentioned in the revised Conclusions and revised Limitations). As we have stated in the revised Conclusions, the “Current results should be validated in another large population of cardiac arrest patients”. We have now also analysed sensitivity, specificity, PPV and NPV of the MRI components and the added clinical examinations (NSE, motor score and pupillary reactions) as was also suggested by other reviewers and are included in Table 2 and Figure 3. In addition absolute values of NSE per group in mean(SD) are presented in eTable 4 in the revised supplement. Furthermore, under revised limitations we address the moderate sample size in a selected (although consecutively enrolled) group of patients with shockable primary rhythm as follows: “First, our results represent a two-center cohort (single country) of a moderate sample size in patients in whom a shockable rhythm was the initial rhythm at time of resuscitation. Therefore, further validation of the results is required in larger number of patients with both shockable and non-shockable primary rhythms.” In addition, the following limitation is now described in the revised limitations as follows: “Fourth, our model was limited by small number of patients with study end point and therefore all variables with predictive value for poor outcome could not be included.” In addition I have the following specific comments:

COMMENT- Why did the authors have mortality as the endpoint and not neurological recovery such as CPC 1-2 compared to CPC 3-5? This important aspect is not properly discussed. The authors report also having MRS scores but the analysis is for mortality, please make a case why this plan is chosen.
RESPONSE: We agree and therefore mRS dichotomization as an outcome endpoint is now used instead of mortality in the revised manuscript. Therefore, all results presented in tables and figures are revised accordingly.

COMMENT: - How did the authors decide on the variables included in the multivariate analysis in Table 2? As seen from Table there were multiple other factors associated with mortality.
RESPONSE: 31 patients had poor (mRS 3-6) neurological outcome. Therefore, this study was limited by a small number of study end point and therefore all factors associated with poor neurological outcome could not be included in the model. In addition, current parameters of MRI and NSE were already predetermined in the protocol. As was also requested by the reviewers we analysed prognostic value of MRI parameters and NSE separately and in combination with pupillary light reflex and motor score. Therefore, the predetermined aim was not to develop the best prediction model based on clinical characteristics such as frequency of epileptic seizures or ROSC. EEG was recorded only if there was a clinical indication based on neurological status and also based on the standard prognostication protocol (please see details in eAppendix in the revised supplement). To avoid overfitting, we adjusted analysis only for the four variables, i.e. for age, sex, study group and site similarly as in our earlier publication (Laitio, JAMA, 2016).

COMMENT:- The study design and the timing of the MRI:s are good for the primary study but not ideal for a study on prognostication. Therefore, it is likely that at the time of the MRI scanning some patients were already conscious but perhaps kept sedated due to the upcoming MRI. In these patients one may question whether there is a need for an MRI at all in clinical practice? This represents a challenge for all studies such as this, ideally only the ones in whom the prognosis is unclear (those unconscious at 48-72 hours) would be the specific group where there is a need for an MRI? Do the authors have the timing when the patient had a GCS motor score of six?
RESPONSE: We agree and this issue was not clearly described in the original manuscript. Results of motor score and timing when patients started to obey commands are now included in the revised Results and Table 2. All patients were comatose at hospital arrival and during hypothermia treatment. After rewarming was completed regular sedation interruptions were commenced in all patients.
It was also predetermined in the protocol that patients had to be kept intubated and sedated until the first MRI was performed.
Under Procedures in the revised Methods (Line 148-150) we have added as follows:
“MRI imaging was scheduled to be performed within 16 hours of rewarming i.e. 36-52 hours after OHCA. Patients were kept intubated and sedated (with sedation interruptions after completion of rewarming) until brain imaging was performed regardless of the neurological status. “

In the revised Results (Line 217-221) we have added as follows:
“Nine of the 61 patients with good neurological outcome and 27 out of 31 patients with poor neurological outcome had motor score ≤ 3 at 72 hours after OHCA (Table 2). Ten patients responded appropriately to commands within 48 hours after OHCA; a further 32 patients were responsive to commands between 48 to 72 hours and 25 patients achieved this state later than 72 hours. Twenty-five never achieved a motor score of 6, all of whom all died.”
We agree that in normal clinical practice MRI may not be necessary if patient starts to obey commands within 48-72 hours as is clearly stated in the recent guidelines. However, our approach of including all enrolled patients regardless of motor score should not compromise an evaluation of prognostic accuracy of current methods per se.

COMMENT- Ideally, the value of MRI should be analyzed together with other markers or poor outcome available at the time point when the investigation is performed. Factors such as brain stem reflexes and NSE would be of interest.
RESPONSE: We agree and in the revised manuscript, we have tried to address all the

concerns raised by the editor and reviewers to improve the ability of this study to provide reliable prognostic information in an appropriate clinical context. It is also clear that current results must be confirmed in another population of cardiac arrest patients (also mentioned in the conclusions and limitations).
In the revised manuscript, the data of NSE, pupillary reactions and motor score within the first 72 hours after OHCA are now included and analysed together with MRI parameters as was also requested by other reviewers. After adding these results we are now able to provide better understanding how the MRI parameters can fit in relation to other commonly used clinical prognostic methods. These results are now presented in Table 2 and in revised figure 3 with new ROC curves. Results of the diagnostic tests (sensitivity, specificity, PPV and NPV) are presented in the revised Table 2.
After rewarming had been completed sedation interruptions were commenced. Motor score and pupillary light reflexes were assessed in all patients during sedation interruptions. This is now also discussed in the revised Discussion (Lines: 240-253) as follows:
“A single measure motor score of ≤ 3 at 72 hours provided the best prognostic value for poor neurological outcome as compared with other methods; NSE at 72 hours was next best. In earlier studies, a cut-off value ≤ 2 for motor score have revealed low specificity and high sensitivity between 70- 80% [20, 21]. Here we demonstrate similar sensitivity values with higher specificity; improvement in the latter attribute can be explained by homogenous cardiac arrest population that only included patients with a shockable primary rhythm. Current AUC of 0.85 for NSE is consistent with the values of 0.86 and 0.90 recorded in earlier large studies in TTM-treated patients [22, 23]. However, comparing NSE results among studies may be problematic because cut-off values vary and a consistent threshold limit for 0% false positive ratio has not been recommended [4]. Higher NSE cut-off values predict worse outcome with cut-off values of ≥23 as compared with current cut-off value of 21 [4, 23]. Among clinical tests in this study pupillary light reflex provided the lowest prognostic value although sensitivity and specificity were similar as in two recent large multicenter studies [11, 24]. One explanation may be that in this study the best value of the first 72 hours was used in order to obtain a value for each patient. As a result, in some patients only assessments of the first 48 hours were applicable which may have led to higher false positive ratio.”

COMMENT- Rather than just reporting the AUC values, I would recommend reporting the sensitivity, specificity, PPV and NPV of the MRI components.
RESPONSE: We agree and these results are now included in the revised manuscript. Please see also our responses above to other reviewers and revised Table 2.

COMMENT- Much of the methods include a thorough discussion of the Xenon trial itself. This is of less interest as this is presented as a prognostic study. Instead the Materials and methods should outline the prognostication algorithm used during the study.
RESPONSE: We agree and the following information is now provided in the revised Methods (Line 148-158):
“MRI imaging was scheduled to be performed within 16 hours of rewarming i.e. 36-52 hours after OHCA. Patients were kept intubated and sedated (with sedation interruptions after completion of rewarming) until brain imaging was performed regardless of the neurological status. A predetermined prognostication protocol (eAppendix in the Supplement) was used to preclude premature decisions to withdraw life-sustaining therapy. DTI and 1H-MRS results did not inform the outcome prognostication. The clinical outcome was evaluated at six months after OHCA with modified ranking scale (mRS) by experienced neurologists.
After rewarming was completed sedation interruptions were initiated and performed every 6 to 12 hours throughout intensive care stay. Motor score of the Glasgow Coma Scale and standard pupillary light reflex were assessed during each sedation interruption by either trained intensive care nurse or on-duty intensive care physicians. NSE serum concentration (Immuno-Electro-Chemi-Luminescent assay, Roche Diagnostics GmbH, Mannheim, Germany) was determined at hospital arrival, and at 24 hours, 48 hours and 72 hours after OHCA.”
In addition, we have removed some of the irrelevant information of the xenon intervention as is described under the line by line responses on pages 7 and 8 of this response letter.

Prognostic value of early phase 1H magnetic resonance spectroscopy and diffusion tensor imaging in combination with neuron-specific enolase and motor score in comatose survivors of out-of-hospital cardiac arrest – A Sub-study of the Xe-Hypotheca Trial

Short Title: Prognostication after cardiac arrest

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Word count 2968

Number of tables 2

Number of figures 3

Number of references 31

Abstract

Purpose

Guidelines recommend brain imaging for neurological prognostication after an out-of-hospital cardiac arrest (OHCA). We aimed to evaluate the predictive accuracy of diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy (1H-MRS) combined with selected clinical examinations and neuron-specific enolase (NSE) for poor neurological outcome after OHCA.

Methods

This study was a two-centre randomised phase 2 clinical drug trial. 110 comatose OHCA patients were randomised to receive either therapeutic hypothermia treatment alone or inhaled xenon in combination with hypothermia, each for 24 hours. The predictive accuracy of DTI, 1H-MRS, motor score, NSE and standard pupillary light reflex for poor neurological outcome (mRS 3−6) at six months were assessed by area under the receiver operating characteristic (ROC) curve.

Results

Of the randomised patients 92 had a complete set of DTI, 1H-MRS and mRS data. The brain imaging was performed in a median (IQR) time of 53 hours (47−64) after OHCA. At six-months, 31 patients had mRS 3−6. The area under ROC curve was 0.73 (95% CI 0.62−0.84) for mean white matter fractional anisotropy, 0.78 (95% CI 0.68−0.88) for ratio of total N-acetylaspartate over total creatine (tNAA/tCr) in basal ganglia, and 0.97 (95% CI 0.94−1.00) for fractional anisotropy and tNAA/tCr combined with NSE and motor score at 72 hours for mRS 3−6.

Conclusions

The combination of mean fractional anisotropy, tNAA/tCr, NSE and motor score within 72 hours had high prognostic accuracy for poor neurological outcome at six months after OHCA. Current result warrants confirmation in another population of cardiac arrest patients.

Keywords: Diffusion tensor imaging, 1H−MRS, Neuro-specific enolase, Motor score, Cardiac arrest, Prognostication

Trial Registration: ClinicalTrials.gov Identifier: NCT00879892

Take-home message: The combination of mean fractional anisotropy, spectroscopy from brain magnetic resonance imaging, NSE and motor score within 72 hours after out-of-hospital cardiac arrest had high prognostic accuracy with an AUC of 0.97 for poor neurological outcome at six months.

Tweet: Guidelines recommend brain magnetic resonance imaging for neurological prognostication after an out-of-hospital cardiac arrest. However, the predictive accuracy of the method is indeterminate. We evaluated the prognostic value of fractional anisotropy, proton magnetic resonance spectroscopy (1H−MRS) from magnetic resonance images, motor score, neuron-specific enolase, pupillary light reflex for poor neurological outcome (mRS 3−6) in survivors of cardiac arrest. Altogether 110 comatose out-of-hospital cardiac arrest patients with ventricular fibrillation as primary rhythm were randomised to receive either standard intensive care treatment alone or inhaled xenon in combination with normal treatment for 24 hours. The brain imaging was performed in a median time of 53 hours after cardiac arrest. The combination of mean fractional anisotropy, 1H-MRS, motor score and neuron-specific enolase within 72 hours after cardiac arrest had high prognostic accuracy with an AUC of 0.97 for poor neurological outcome.

Introduction

In-hospital mortality of successfully resuscitated out-of-hospital cardiac arrest (OHCA) patients remains high, ranging from 41% to 86%, despite implementation of therapeutic hypothermia (also referred to as targeted temperature management) and other improvements in the treatment of these patients [1, 2]. The dominant cause of morbidity and mortality in survivors of OHCA is hypoxic-ischemic brain damage and survivors have a high risk for a diverse spectrum of neurological injuries [3].

Based on the current guidelines neurological prognostication is recommended in patients who exhibit an extensor motor response to pain at 72 hours or later after cardiac arrest [4]. A multimodal approach with a combination of clinical assessment, serum biomarkers, electroencephalography, somatosensory evoked potentials and neuroimaging is recommended during the early phase at 3−5 days after cardiac arrest. However, a poor outcome cannot be predicted with certainty and the assessment may be confounded by contradictory results from the different modalities [4, 5]. Therefore, there is an unmet need for new biomarkers to improve the accuracy of early-phase prognostication in order to identify patients with poor neurological outcome.

Among conventional methods of neuroimaging the value of diffusion weighted imaging (DWI) in neurological prognostication is well-demonstrated, but it has been reported to underestimate the extent of ischemic injury during the first three days after OHCA [6]. While grey matter has classically been thought to be more sensitive to hypoxic-ischemic brain damage, white matter is also highly vulnerable even in the early stages of ischemia [7]. Diffusion tensor imaging (DTI) is an extension of DWI that allows evaluation of microstructural integrity of brain white matter using directional assessment of water diffusion, thus potentially being more sensitive than DWI to detect white matter damage in OHCA patients.

We have previously demonstrated that impaired white matter micro-integrity, reflected by lower fractional anisotropy in non-survivors than in the survivors, was caused by demyelination in the early phase after OHCA [8]. Proton magnetic resonance spectroscopy (1H−MRS) is another advanced magnetic resonance technique with some evidence of prognostic value in hypoxic-ischaemic brain damage in adults after stroke and cardiac arrest as well as in asphyxiated neonates [9-11]. However, the value of these imaging indices, either alone or in combination with motor score, neuron-specific enolase (NSE) and pupillary light reflex, for predicting poor neurological outcome at early phase after OHCA, has yet to be established.

As defined in the original study protocol, the purpose of this study was to compare the predictive values for 6-month neurological outcome, dichotomized as good (mRS 0−2) and poor (mRS 3−6), of fractional anisotropy

from DTI, with several brain metabolites from 1H−MRS each obtained by MRI along with motor score, NSE and pupillary light reflex performed in comatose survivors within 72 hours after OHCA.

Methods

Study Design

Xe-HYPOTHECA trial (ClinicalTrials.gov NCT00879892) was a randomized 2-group single-blinded phase 2 clinical drug trial at two multipurpose intensive care units (ICU) in Finland. The study was approved by the ethics committee of the Hospital District of Southwest Finland and the institutional review boards of the Helsinki University Hospital and the Finnish Medicines Agency. All patients' next of kin or legal representative gave written informed assent within 4 hours after hospital arrival. Consent was sought from patients if they regained consciousness. As described earlier, an independent data and safety monitoring committee reviewed data after enrolment of every 4 patients and after each 6-month interval [8]. The study was conducted according to good clinical practice and the latest revision of the Declaration of Helsinki. Study design and methodology was consistent with the STARD guidelines for reporting diagnostic accuracy studies [12].

Participants

Consecutive comatose survivors of witnessed out-of-hospital cardiac arrest from an initial shockable rhythm admitted to the Turku and Helsinki University hospitals between August 2009 and September 2014 were screened for eligibility. Detailed inclusion and exclusion criteria are listed in eAppendix in the Supplement. We have previously reported the primary and secondary clinical end points of the Xe-HYPOTHECA trial [8, 13]. The protocol of the Xe-HYPOTHECA trial has also been published [8].

Randomisation and Blinding

The patients were allocated in a 1:1 ratio with random block sizes of 4, 6, and 8 to receive either therapeutic hypothermia treatment alone for 24 hours or inhaled xenon (LENOXe, Air Liquide Medical GmbH, Düsseldorf, Germany) in combination with hypothermia for 24 hours as described earlier [8]. The neurological end-point evaluators as well as the patients were blinded to the treatment.

Procedures

MRI imaging was scheduled to be performed within 16 hours of rewarming i.e. 36 to 52 hours after OHCA. Patients were kept intubated and sedated (with sedation interruptions after completion of rewarming) until brain imaging was performed regardless of the neurological status. A predetermined prognostication protocol (eAppendix in the Supplement) was used to preclude premature decisions to withdraw life-sustaining therapy.

DTI and 1H-MRS results did not inform the outcome prognostication. The clinical outcome was evaluated at six months after OHCA with modified ranking scale (mRS) by experienced neurologists.

After rewarming was completed sedation interruptions were initiated and performed every 6 to 12 hours throughout intensive care stay. Motor score of the Glasgow Coma Scale and standard pupillary light reflex were assessed during each sedation interruption by either trained intensive care nurse or on-duty intensive care physicians. NSE serum concentration (Immuno-Electro-Chemi-Luminescent assay, Roche Diagnostics GmbH, Mannheim, Germany) was determined at hospital arrival, and at 24 hours, 48 hours and 72 hours after OHCA.

Siemens Magnetom Verio 3T scanner (Siemens Medical Solutions, Erlangen, Germany) with 12-element Head Matrix coil was used in both MRI centres. DTI and DWI data were acquired using diffusion weighted spin-echo echo planar imaging (SE−EPI) sequence with 20 diffusion encoding directions (see eTable1 in the Supplement for details).

FSL software library (version 6.0, Analysis Group, FMRIB, Oxford, United Kingdom) was used for processing the DTI images, following the tract-based spatial statistics (TBSS) processing pipeline [14, 15]. This observer-independent and hypothesis-free method has the ability to spatially locate group differences in the DTI data. Mean fractional anisotropy value of white matter was calculated as a mean value of all the voxels in the skeleton, and mean diffusivity (MD) of the basal ganglia was assessed from the DWI dataset (see eMethods in Supplement for details).

1H−MRS data were acquired from the region of basal ganglia by utilising Chemical Shift Imaging (CSI) technique (see eTable 2 for details). Acquired data were analysed using the LCModel software (version 6.3−0C) [16]. An average of all analysed voxels, except the ones containing cerebrospinal fluid (CSF) were selected for the final analysis (see eFigure 1). The metabolite concentration values were corrected for relaxation effects (eMethods) but absolute concentration values were not feasible to use. Therefore, the amount of tNAA and total choline were expressed as ratios over total creatine, i.e. tNAA/tCr and tCho/tCr, as it is expected to remain stable. In addition, tNAA/tCho ratio was presented as both the amount of tNAA and tCho is considered to be related to neuronal density, activity and integrity [17].

Statistical analysis

The sample size of 110 patients was based on a power analysis of the fractional anisotropy values from brain magnetic resonance imaging, i.e. the primary end-point of the Xe-HYPOTHECA trial [8]. The categorical demographic data and baseline clinical characteristics between groups of mRS 0−2 and mRS 3−6 were

compared with chi-square or Fisher's exact test. Two-sample t-test or Mann−Whitney U−test was used to test the differences in continuous demographic data and baseline clinical characteristics between the groups mRS 0−2 and mRS 3−6. The normality of continuous variables was evaluated visually using histograms. The mean differences in mean fractional anisotropy, mean diffusivity of the basal ganglia and 1H−MRS data and NSE at 48 and 72 hours after OHCA between the groups were tested with two-sample t-test. Age-, sex-, treatment-, and site-adjusted mean differences between the groups were compared with analysis of covariance. NSE values were log-transformed for statistical analysis due to positively skewed distribution. The associations of the mean fractional anisotropy, mean diffusivity of basal ganglia, 1H−MRS and NSE values with 6-month mortality were analysed by using Cox regression analysis after adjustment for age, sex, treatment, and site. The follow-up time for survival analysis was calculated from the time of cardiac arrest until death or 6 months. The observation was censored in the survival analysis if the patient was withdrawn from the study or was still alive at the end of the 6-month follow-up. Permutation-based voxel-wise statistical analysis with tract-based spatial statistics in conjunction with family-wise error correction was used for multiple comparisons across space to obtain group differences in the white matter tracts [8, 14, 15].

The results are expressed using adjusted hazard ratios (HR) with 95% confidence intervals (CI). The prognostic values of fractional anisotropy, tNAA/tCr, NSE and motor score at 72 hours after OHCA and logistic regression derived combined model (eMethods) were evaluated by calculating the area under the curve (AUC) of receiver operating characteristic (ROC) curve using a nonparametric method. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for each prognostic variable were calculated. Optimal cut-off values were chosen by using Youden Index (sensitivity + specificity−1).

A 2-sided p-value less than 0.05 was considered statistically significant. Statistical analyses were performed with SAS System for Windows, version 9.4 (SAS Institute Inc., Cary, NC) and SPSS Statistics for Macintosh, version 24 (IBM Corp., Armonk, NY). The hazard ratio plot was created using DistillerSR Forest Plot Generator (Evidence Partners, Ottawa, Canada)

Results

Patients

Of the 224 patients screened for eligibility for the Xe-HYPOTHECA trial, 110 were included. Of these, 97 underwent magnetic resonance imaging in a median (inter-quartile range) time of 53 hours (47−64) after OHCA and 93 had 1H−MRS, DTI and DWI data available (Fig. 1).

One patient was withdrawn six days after the index event by the next of kin and therefore 92 out of 93 had applicable mRS data (Fig. 1, eTable 3). At six months after OHCA, 61 patients had good (mRS 0−2) and 31 patients had poor (mRS 3−6) neurological outcome. Patient demographics and clinical characteristics are presented in table 1.

Pupillary light reflex, NSE and motor score

Pupillary light reflex within 72 hours did not differ. NSE at 48 and 72 hours after OHCA were significantly higher in patients with poor neurological outcome than in the patients with good neurological outcome at six months (Table 2, eTable 4). Nine of the 61 patients with good neurological outcome and 27 out of 31 patients with poor neurological outcome had motor score ≤ 3 at 72 hours after OHCA (Table 2). Ten patients responded appropriately to commands within 48 hours after OHCA; a further 32 patients were responsive to commands between 48 to 72 hours and 25 patients achieved this state later than 72 hours. Twenty-five patients never achieved a motor score of 6, of whom all died.

DTI, DWI and 1H−MRS results

Mean fractional anisotropy values of the DTI, and tNAA/tCr and tNAA/tCho ratios of the 1H−MRS were significantly higher in patients with mRS 0−2 than in patients with mRS 3−6 (Table 2). Results of the tract-based spatial statistics analysis are visualized with a statistical parametric map (Fig. 2). Results of the age-, sex-, treatment-, and site-adjusted survival analyses are presented in eFigure 2 in the supplement.

Receiver operating characteristic analysis and diagnostic tests

Both motor score and NSE at 72 hours had better diagnostic values than mean diffusivity, fractional anisotropy or 1H−MRS measures for poor neurological outcome (Table 2, Fig 3). There was no significant difference (P=0.53) in predictive power between fractional anisotropy and tNAA/tCr. Area under the curve was 0.92 (95 %

CI 0.86–0.97) for a combination of fractional anisotropy, NAA/Cr and NSE and 0.97 (95% CI 0.94–1.00) when FA and tNAA/tCr were combined with NSE and motor score at 72 hours after OHCA (Fig 3).

Discussion

The main finding of this study was that mean fractional anisotropy of white matter and tNAA/tCr of the basal ganglia in combination with NSE and motor score at 72 hours had high prognostic value for poor neurological outcome at six months after OHCA. Quantitative analysis of the mean diffusivity of the basal ganglia or standard pupillary light reflex did not provide any independent prognostic value.

A single measure motor score of ≤ 3 at 72 hours provided the best prognostic value for poor neurological outcome as compared with other methods; NSE at 72 hours was the next best. In earlier studies, a cut-off value ≤ 2 for motor score have revealed low specificity and high sensitivity between 70−80% [20, 21]. Here we demonstrate similar sensitivity values with higher specificity; improvement in the latter attribute can be explained by homogenous cardiac arrest population that only included patients with a shockable primary rhythm. Current AUC of 0.85 for NSE is consistent with the values of 0.86 and 0.90 recorded in earlier large studies in TTM-treated patients [22, 23]. However, comparing NSE results among studies may be problematic because cut-off values vary and a consistent threshold limit for 0% false positive ratio has not been recommended [4]. Higher NSE cut-off values predict worse outcome with cut-off values of ≥23 as compared with current cut-off value of 21 [4, 23]. Among clinical tests in this study pupillary light reflex provided the lowest prognostic value although sensitivity and specificity were similar as in two recent large multicenter studies [11, 24]. One explanation may be that in this study the best value of the first 72 hours was used in order to obtain a value for each patient. As a result, in some patients only assessments of the first 48 hours were applicable, which may have led to higher false positive ratio.

Fractional anisotropy is a DTI-derived scalar value that reflects white matter tissue characteristics such as fiber density, organization coherence, myelination, and axon diameter [25]. Lower fractional anisotropy values in ischemic white matter probably represent a combination of myelin damage, axonal degeneration, and oedema, which all contribute to loss of directional diffusion in white matter tracts [26].

A very recent study demonstrated a prognostic value of decreased mean global white matter fractional anisotropy levels imaged 7 to 28 days after cardiac arrest for long-term neurological outcome with an area under ROC of 0.95 in a subset of 150 patients with a persistent unresponsiveness at day 7 [11]. According to earlier evidence most survivors regain consciousness within a week and usually all of them within 10 days after cardiac arrest [4, 5, 19, 27, 28]. However, Velly and colleagues revealed that as many as 22% of patients who were without a response to simple commands a week after cardiac arrest may still have a favourable outcome at six months after cardiac arrest [11]. Current lower prognostic value for fractional anisotropy at early subacute phase

can be partly due to evolving white matter injury over time leading to distinctive predictive value for specific imaging time windows [29].

In our study, DWI of the basal ganglia alone was a poor predictor of neurological outcome. Previous studies have shown that some patients with poor outcome have no DWI signal changes in the basal ganglia [30], and some patients with even severe diffusion restriction in the basal ganglia may end up with good neurological recovery [11]. This may explain the poor predictive value in our study. The underlying physiological reason for this phenomenon remains unknown.

NAA is ubiquitous in the central nervous system, and is a marker of the integrity of mature neurons; persistent reductions in NAA have been used as a marker of neuronal loss and extent of neurological damage and have prognostic value for outcome in stroke and cardiac arrest [9, 11, 31]. These earlier interpretations were supported by the present study revealing that ratios of tNAA/tCr and tNAA/tCho in the basal ganglia are independent predictors for poor neurological outcome at six months.

In this study, a combined model of fractional anisotropy, tNAA/tCr, NSE and motor score provided high prognostic value with AUC of 0.97 as early as 72 hours for all consecutively enrolled OCHA patients with ventricular fibrillation as a primary rhythm. As a strength of the current new model fractional anisotropy, tNAA/tCr and NSE are observer independent methods and provided high accuracy even without motor score. Although motor score, NSE and combination of fractional anisotropy and tNAA/tCr provided good prognostic values, none could be used as a singular method due to insufficient accuracy. These results warrant further confirmation in another population including also patients with asystole and PEA as primary rhythm.

Limitations

There are some limitations in this study. First, our results represent a two-center cohort (single country) of a moderate sample size in patients in whom a shockable rhythm was the initial rhythm at time of resuscitation. Therefore, further validation of the results is required in larger number of patients with both shockable and non-shockable primary rhythms. Second, metabolite concentration ratios instead of absolute values or inter-subject metabolite concentrations were used. The use of absolute concentration values would have required the use of reference solutions for calibrations and information about coil loading and T2 attenuation, or the use of a water-referencing method; none of these options were available for this study. In routine clinical practice, absolute metabolite values are seldom available and thus this approach was not deemed feasible. Third, due to a possible bias caused by the neuroprotective effect of the xenon on the prognostic value of the current metrics all statistical analyses were adjusted with the treatment group. Fourth, our model was limited by small number of

patients with study end point and therefore all variables with predictive value for poor outcome could not be included.

Conclusions

In summary, fractional anisotropy of DTI and tNAA/tCr of 1H−MRS in early subacute phase combined with NSE and motor score at 72 hours have high prognostic accuracy for poor neurological outcome in OHCA patients with ventricular fibrillation as a primary rhythm. Current results should be validated in another large population of cardiac arrest patients.

Author Contributions: Dr Laitio T had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Laitio Timo, Laitio Ruut, Parkkola Riitta, Saunavaara Jani, Virtanen Sami
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Virtanen Sami and Koskensalo Kalle wrote the first draft
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Vahlberg Tero
Obtained funding: Laitio Timo and Roine O. Risto
Administrative, technical, or material support: Laitio Timo
Study supervision: Laitio Timo, Parkkola Riitta

Funding: The study was funded by Academy of Finland and by State Research Funding.

Role of the funder: The funding organizations did not have any role in design and conduct of the study, collection, management, analysis, and interpretation of the data and preparation, review or approval of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Acknowledgements: We thank research nurses Keijo Leivo (Registered nurse, Turku University Hospital, was compensated for his contribution) and Tuukka Tikka (Registered nurse, Helsinki University hospital, was compensated for his contribution) for taking care of the logistics of this study.

Conflict of Interest Disclosures: TL is a member of the Trial Executive Committee for XePOHCAS, a phase 3 RCT designed to test the efficacy and safety of patients with post-cardiac arrest syndrome. The XePOHCAS trial is sponsored by NeuroproteXeon. TL reports grant from Finnish Foundation for Cardiovascular Research. RL is a paid governmental consultant official for the National Supervisory Authority for Welfare and Health. AS reports grants from Academy of Finland and Finnish Foundation for Cardiovascular Research during the conduct of the study; consulting fees from GE healthcare, Novartis, Abbot, and Astra Zeneca. Dr. Maze is a founder, board director, and equity shareholder of NeuroproteXeon, a company that intends to commercialize the use of xenon for ongoing acute neurological injury, including its administration to successfully resuscitated patients after out-of-hospital cardiac arrest. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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Figure legends

Fig. 1

Flow diagram

Flow of the participants in the study Prognostic value of early phase 1H magnetic resonance spectroscopy and diffusion tensor imaging in combination with neuron-specific enolase and motor score in comatose survivors of out-of-hospital cardiac arrest – A Sub-study of the Xe-Hypotheca Trial**.**

Fig. 2

Whole brain visualisation of the results of cerebral white matter damage

White matter damage leads to a loss of microstructural organization that can be quantified by the loss of directionality in the diffusion of water molecules in the white matter tracts. Fractional anisotropy is a scalar value representing this directionality of water diffusion; lower fractional anisotropy values are indicative of less organized diffusion and are an index of more extensive white matter damage. The visualization presents the results of the voxel-wise tract-based spatial statistics analysis of fractional anisotropy values between patients with good (mRS 0−2) and poor (mRS 3−6) 6-month neurological outcome. Voxels with significantly ($P < 0.05$, family-wise error corrected for multiple comparisons) lower fractional anisotropy values in patients with poor neurological outcome were identified and are shown in red in the statistical visualization (i.e., 78.2% of all 123994 analysed voxels), whereas the areas in which there was no significant difference in fractional anisotropy values between the groups are shown in green (i.e., 21.8% of all analysed voxels).

According to the Johns Hopkins University white matter tractography atlas [18], the tract-wise distribution of the voxels (percentages in parentheses below) with significantly ($P<0.05$; family-wise error corrected for multiple comparisons) lower fractional anisotropy in non-survivors (marked red in the figure) were as follows: cingulum (cingulate gyrus) (88.0%), cingulum (hippocampal region) (81.4%), forceps minor (92.0%) and major (71.7%), superior longitudinal fasciculus (85.3%), inferior longitudinal fasciculus (80.5%), anterior thalamic radiation (85.1%), inferior fronto-occipital fasciculus (84.7%), corticospinal tract (79.9%), uncinate fasciculus (89.3%), and the body of corpus callosum (98.0%).

Fig. 3

Receiver operating characteristic curves

Receiver operating characteristic curves of fractional anisotropy combined with tNAA/tCr, fractional anisotropy combined with NAA/tCr and NSE at 72 hours, and the combination of fractional anisotropy, tNAA/tCr, NSE and motor score at 72 hours.

Figure 1

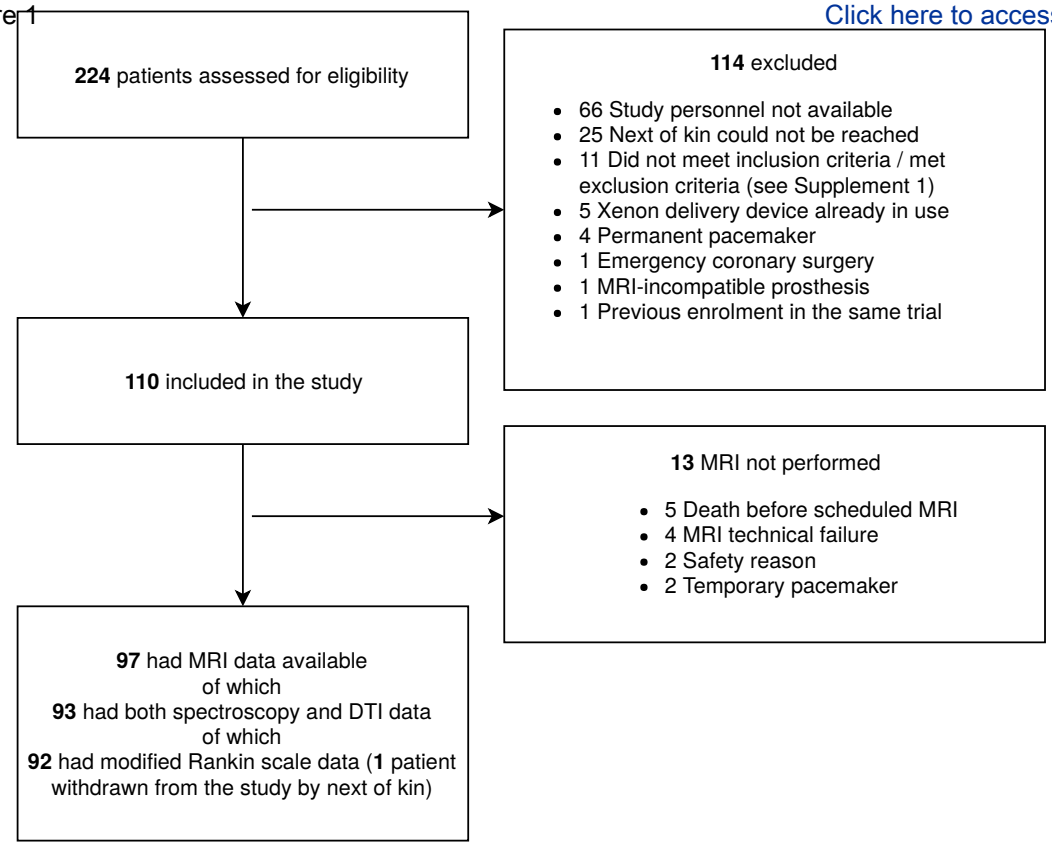

Figure 2

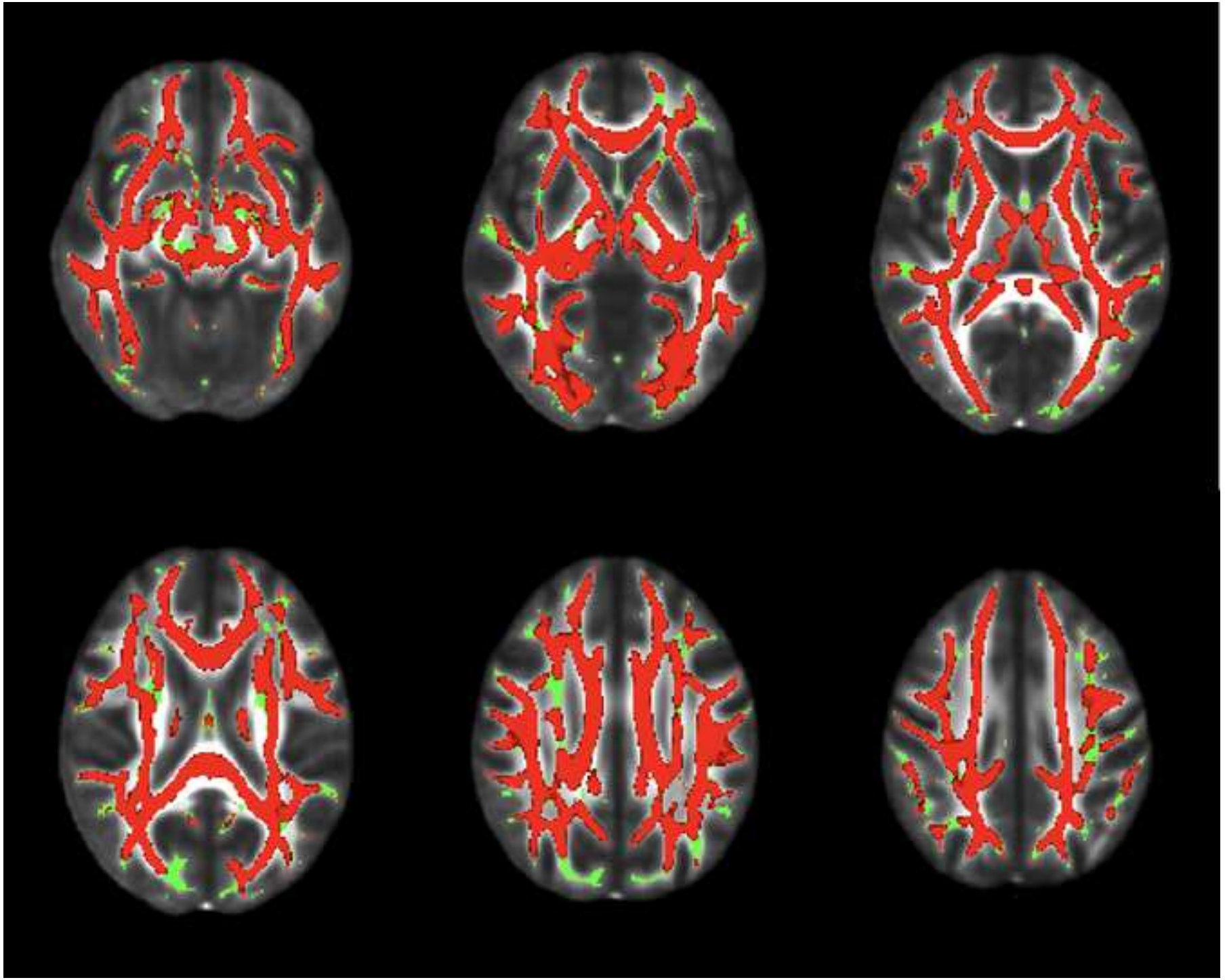

Figure 3

Click here to access/download;Figure;Figure 3revised.eps

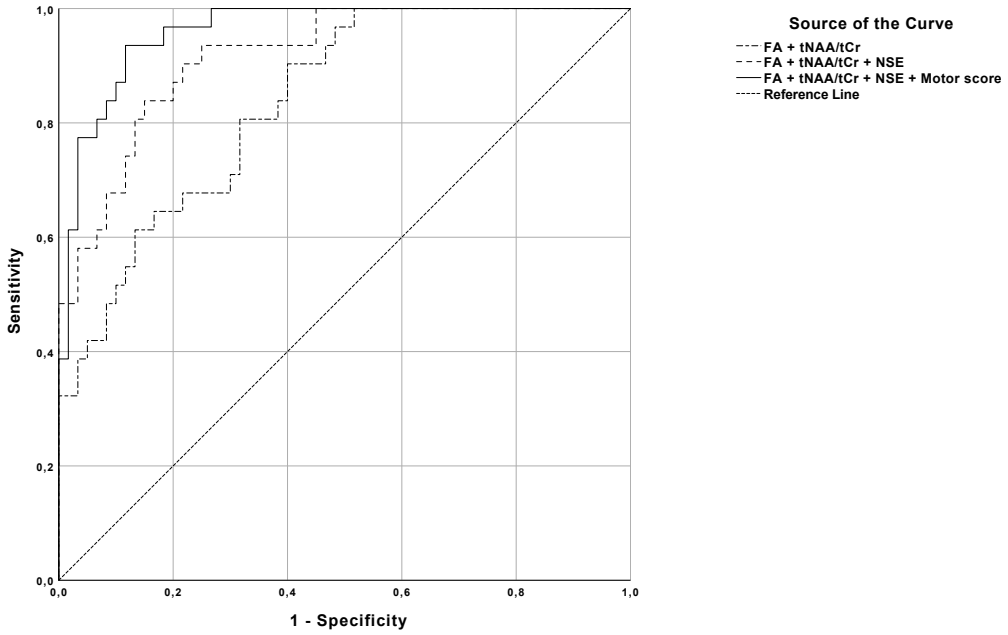

Table 1. Demographic data and clinical characteristics of the patients.

	All **n = 93**	**mRS 0-2** **n = 61**	**mRS 3-6** **n = 31**	**P-value**
Baseline characteristics				
Age, y, median (IQR)	61.0 (54.5−67.0)	58.0 (53.0−64.0)	64.0 (57.0−71.0)	0.0029
Female sex, n (%)	24 (25.8)	43 (70.5)	6 (19.4)	0.2945
Coronary artery disease, n (%)	70 (75.3)	44 (72.1)	26 (83.9)	0.2121
Hypertension, n (%)	42 (45.2)	27 (44.3)	15 (48.4)	0.7073
Congestive heart failure, n (%)	7 (7.5)	4 (6.6)	3 (9.7)	0.6842
Diabetes, n (%)	13 (14)	6 (9.8)	7 (22.6)	0.1190
Asthma or chronic obstructive pulmonary disease, n (%)	13 (14)	9 (14.8)	4 (12.9)	1.000
Dyslipidemia, n (%)	35 (37.6)	20 (32.8)	14 (45.2)	0.2451
Smoker, n (%)	35 (37.6)	21 (35.6)	14 (45.1)	0.3763
Previous stroke, n (%)	21 (22.6)	11 (18.0)	20 (64.5)	0.1244
Selected laboratory values				
pH, median (IQR)	7.32 (7.29−7.37)[a]	7.33 (7.30−7.39)[c]	7.32 (7.27−7.35)[f]	0.1132
Lactate, µmol/l, median (IQR)	1.94 (1.30−2.80)[b]	1.80 (1.15−2.70)[d]	2.50 (1.55−3.24)[f]	0.0922
Creatine, µmol/l, median (IQR)	88.0 (77.0−111.8)[a]	85.0 (74.0−103.0)[e]	101.5 (83.0−145.0)[g]	0.0046
Resuscitation details				
Bystander resuscitation, n (%)	67 (72)	46 (75.4)	20 (64.5)	0.2727
Delay in EMS, min, mean (SD)	8.6 (3.4)	8.7 (3.1)	8.2 (4.1)	0.5041
ROSC, min, mean (SD)	22.0 (6.8)	20.2 (6.3)	25.3 (6.7)	0.0005
No flow, min, median (IQR)	0.0 (0.0−4.0)	0.0 (0.0−0.0)	0.0 (0.0−6.0)	0.2546
Cooling procedure details				
Core temperature before start of cooling, °C, mean (SD)	35.04 (1.25)	35.13 (1.26)	34.8 (1.26)	0.3088
Time from OHCA to target temperature, min, median (IQR)	311 (263−370)	295 (246−354)	354 (291−406)	0.0194

Cooling rate, °C /h, median (IQR)	0.42 (0.25−0.50)	0.43 (0.29−0.56)	0.36 (0.14−0.47)	0.263
Clinical characteristics during ICU stay				
Status epilepticus, n (%)	26 (28.0)	6 (9.8)	19 (61.3)	< 0.0001
STEMI, n (%)	34 (36.6)	19 (31.1)	15 (48.4)	0.1054
NSTEMI, n (%)	54 (58.1)	37 (60.7)	16 (51.6)	0.4068
Acute kidney injury, n (%)	20 (21.5)	12 (19.7)	8 (25.8)	0.5001
Xenon group, n (%)	47 (50.5)	31 (50.8)	15 (48.4)	0.8254
Norepinehrine cumulative dose of 72h after ICU admission, mg, median (IQR)	16.5 (6.8−33.8)	13.5 (6.0−30.2)	30.4 (17.9−45.1)	0.0017

Data are expressed as number (percentage) unless otherwise indicated.

Due to missing data mRS was applicable for N=92.

Due to missing data laboratory values are applicable for [a] N=79, [b] N=78, [c] N=55, [d] N=54, [e] N=56, [f] N=24, [g] N=23

Due to missing data pupillary reactivity values are applicable for [h] N=92, [i] N=30

Table 2. Results of magnetic resonance spectroscopy, diffusion tensor and diffusion weighted imaging, neuron-specific enolase, motor score and pupillary light reflex in patients with good and poor neurological outcome at six months after OHCA.

	Unadjusted Mean (SD)		Mean Difference (95% CI)		p value	
	mRS 0−2 (n = 61)	mRS 3−6 (n = 31)	Unadjusted	Adjusted [a]	Unadjusted	Adjusted [a]
tNAA/tCr	2.43 (0.21)	2.18 (0.22)	0.25 (0.15−0.34)	0.22 (-0.12−0.31)	< 0.0001	< 0.0001
tCho/tCr	0.35 (0.04)	0.35 (0.04)	0.01 (-0.01−0.03)	0.01 (-0.01−0.02)	0.3831	0.5403
tNAA/tCho	6.96 (0.91)	6.37 (0.68)	0.59 (0.22−0.96)	0.54 (0.16−0.93)	0.0020	0.0065
Basal ganglia MD, 10^{-6} mm^2/s	807.3 (54.8)	809.8 (87.9)	-2.53 (-32.18−27.13)	3.20 (-27.92−34.31)	0.8659	0.8387
Fractional anisotropy	0.43 (0.02)	0.41 (0.03)	0.03 (0.01−0.04)	0.02 (0.01−0.03)	< 0.0001	0.0014
NSE 48 h [b]	2.97 (0.38)	3.71 (0.71)	-0.73 (-0.96−-0.51)	-0.91 (-1.21−-0.61)	< 0.0001	< 0.0001
NSE 72 h [b]	2.71 (0.48)	3.65 (0.87)	-0.94 (-1.22−-0.66)	-0.91 (-1.21−-0.61)	< 0.0001	< 0.0001
			Unadjusted Odds Ratio (95 CI)	Adjusted [a] Odds Ratio (95 CI)		
GCS motor score 4-6 at 72 h, n(%)	52 (85.3)	4 (12.9)	38.99 (10.9−138.34)	37.31 (9.85−141.32)	< 0.0001	< 0.0001
Pupillary light reflect at 72 h, n(%)	54 (88.5)	22 (73.3)	2.80 (0.91−8.67)	2.28 (0.64−8.09)	0.0734	0.2039

	AUC	95% CI	Cut-off	Sensitivity	Specificity	Positive predictive value	Negative predictive value
tNAA/tCr	0.78	0.68−0.88	≤2.313	0.77	0.69	0.56	0.86
tCho/tCr	0.58	0.45−0.71	≤0.3445	0.61	0.59	0.43	0.75
tNaa/tCho	0.69	0.58−0.80	≤7.154	0.97	0.39	0.45	0.96
Basal ganglia MD	0.54	0.40−0.68	≥882	0.26	0.93	0.67	0.71

Fractional anisotropy	0.73	0.62−0.84	≤0.4235	0.68	0.69	0.53	0.81
NSE 48h	0.84	0.74−0.94	≥25	0.84	0.79	0.67	0.91
NSE 72h[c]	0.85	0.76−0.93	≥21	0.77	0.82	0.69	0.88
GCS motor score at 72h	0.88	0.80−0.96	≤3	0.87	0.85	0.75	0.93
Absent pupillary light reflect at 72 h[d]	0.58	0.49−0.67	NA	0.27	0.89	0.53	0.71
tNAA/tCr+Fractional anisotropy	0.83	0.7 −0.91	NA	0.90	0.59	0.53	0.92
tNAA/tCr+Fractional anisotropy+NSE 72h	0.92	0.86−0.97	NA	0.84	0.85	0.74	0.91
tNAA/tCr+Fractional anisotropy+NSE 72h+GCS motor score at 72h	0.97	0.94−1.00	NA	0.87	0.93	0.87	0.93

[a] Data are adjusted for age, sex, site and treatment group.

[b] Values were log-transformed for statistical analysis

[c] Due to missing data NSE 72h values are applicable for n=60 in mRS 0 − 2 patients

[d] Due to missing data pupillary reactivity values are applicable for n=30 in mRS 3 − 6 patients

Values are mean (SD) unless otherwise indicated. mRS = modified ranking scale; CI = confidence intervals; tNAA = total N-acetyl aspartate; tCho = total choline; tCr = total creatine; mean diffusivity = MD; NSE = neuron-specific enolase; GCS = Glasgow Coma Scale; AUC = the area under the ROC (receiver operating characteristic) curve