

# Comparison of Prognostic Value of Early Phase 1H Magnetic Resonance Spectroscopy and Diffusion Tensor Imaging With Neuron-Specific Enolase at 72 Hours in Comatose Survivors of Out-of-hospital Cardiac Arrest – A Sub-Study of the Xe-Hypotheca Trial

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# Abstract

## Background

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

## Methods

In 92 patients with complete data set, the predictive accuracy of DTI, 1H-MRS, and NSE at 72h for poor neurological outcome (mRS 3-6) at six months were assessed by area under the receiver operating characteristic (ROC) curve as predetermined in the protocol. Additional post-hoc analyses were also performed to find a model with the best predictive power for poor neurological outcome. The brain imaging was performed in a median (IQR) time of 53 hours (47-64) after OHCA.

## Results

At six-months, 31 patients had mRS 3-6, considered to reflect a poor neurological outcome. There was no significant difference between area under ROC curve of 0.73 (95% CI 0.62-0.84) for DTI, 0.78 (0.68-0.88) for 1H-MRS and 0.85 (0.76-0.93) for NSE at 72h for predicting poor outcome at 6 months. In the post-hoc analysis, the combination of DTI, 1H-MRS, and NSE with motor score at 72 hours and epileptic seizures provided the best predictive power with area under ROC curve of 0.98 (95% CI 0.96 – 1.00) as compared to 0.92 (0.86 – 0.97) for DTI, 1H-MRS and NSE at 72 hours alone ( $p = 0.009$ ).

## Conclusions

Neither early stage DTI nor 1H-MRS imaging was better than NSE at 72h in predicting poor outcome in this patient group. Although the addition of neuroimaging to the best model using current clinical and laboratory parameters improved the diagnostic performance, the costs and challenges of MR imaging of critically ill patients may outweigh this small benefit.

## Trial registration

ClinicalTrials.gov NCT00879892. Registered on 13 April 2009.

## Background

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 treatments [1, 2]. The major cause of morbidity and mortality in survivors of OHCA is hypoxic-ischemic brain damage with survivors at 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 assessment 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 a potential poor neurological outcome.

Among conventional methods of neuroimaging the value of diffusion weighted imaging (DWI) in neurological prognostication is well-demonstrated; however, DWI has been reported to underestimate the extent of ischemic injury during the first three days after OHCA [6]. While gray 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 [8]. 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 [9].

Proton magnetic resonance spectroscopy (1H-MRS) is another advanced magnetic resonance technique with some evidence of prognostic value in hypoxic-ischemic brain damage in adults after stroke and cardiac arrest as well as in asphyxiated neonates [10-12].

DTI performed between 7 and 28 days after cardiac arrest has shown great promise to accurately predict neurological outcome [12]. As defined in the original study protocol, the purpose of this study was to test the hypothesis that DTI and/or 1H-MRS could also be used at an earlier phase to assess the neurological outcome in this patient group. We assessed the predictive values for 6-month neurological outcome, dichotomized as good (mRS 0-2) and poor (mRS 3-6), of fractional anisotropy from DTI, several brain metabolites from 1H-MRS each obtained by MRI (magnetic resonance imaging) and neuron-specific enolase (NSE) performed in comatose survivors within 72 hours after OHCA. Additional post-hoc analyses were performed to find a model with the best predictive power for poor neurological outcome at six months 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 when 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 [9]. 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 [13].

## Participants

Consecutive comatose survivors of witnessed OHCA 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 [9, 14]. The protocol of the Xe-HYPOTHECA trial has also been published [9].

## Randomization 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 [9]. 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. 36-52 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 were not available at the time of prognostication or at any time during the intensive care. The clinical outcome was evaluated at six months after OHCA with modified Rankin 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 was 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. An electroencephalogram was recorded only if it was clinically indicated.

Siemens Magnetom Verio 3T scanner (Siemens Medical Solutions, Erlangen, Germany) with 12-element Head Matrix coil was used in both MRI centers. DTI 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 [15, 16]. 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. (see eMethods in Supplement for details).

<sup>1</sup>H-MRS data were acquired from the region of basal ganglia by utilizing Chemical Shift Imaging (CSI) technique (see eTable 2 for details). Acquired data were analyzed using the LCModel software (version 6.3-0C) [17]. An average of all analyzed voxels, except the ones containing cerebrospinal fluid (CSF) were selected for the final analysis (see Figure 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 (total N-acetyl aspartate) 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, apart from the tNAA/tCho ratio, the amount of tNAA and tCho was assessed as these individual parameters are related to neuronal density, activity and integrity [18].

## 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 [9]. 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, <sup>1</sup>H-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, <sup>1</sup>H-MRS and NSE values with 6-month mortality were analyzed 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 [9, 15, 16].

The results are expressed using adjusted hazard ratios (HR) with 95% confidence intervals (CI). The prognostic values of fractional anisotropy, tNAA/tCr and NSE 72 hours after OHCA and logistic regression derived combined models including selected clinical variables were evaluated post-hoc 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 was calculated. Optimal cut-off values were chosen by using the 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 both 1H-MRS and DTI data available (Fig. 2).

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. 2, eTable 3). At six months after OHCA, 61 patients had good (mRS 0-2) and 31 patients had poor (mRS 3-6) neurological functional outcome. Patient demographics and clinical characteristics are presented in Table 1.

### NSE, epileptic seizures and motor score

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). In addition, the number of patients with epileptic seizures, and motor score  $\leq 3$  at 72 hours, and time to return of spontaneous circulation (ROSC) were significantly greater in patients with poor neurological outcome (Table 2). Nine of the 61 patients with good neurological outcome and 27 out of 31 patients with poor neurological outcome had motor score  $\leq 3$  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 patients never achieved a motor score of 6, all of whom died. A single measure motor score of  $\leq 3$  at 72 hours provided the best prognostic value for poor neurological outcome as compared with other methods (Table 2).

### DTI 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. 3). Results of the age-, sex-, treatment-, and site-adjusted survival analyses are presented in Figure 4.

## Receiver operating characteristic analysis and diagnostic tests

The area under ROC curve for predicting poor outcome was 0.73 (95% CI 0.62-0.84) for DTI (FA), 0.78 (0.68-0.88) for 1H-MRS (tNAA/tCr) and 0.85 (0.76-0.93) for NSE at 72h; FA vs tNAA/tCr, FA vs NSE and tNAA/tCr vs NSE;  $p = 0.53$ ,  $p = 0.13$  and  $p = 0.38$ , respectively (Fig. 5). A subgroup of 46 patients had a motor score below 6 at 72 h after OHCA. The area under ROC curve for predicting poor outcome in this subgroup was just slightly smaller than in the whole data: 0.72 (95% CI 0.57-0.87) for DTI (FA), 0.76 (0.62-0.90) for 1H-MRS (tNAA/tCr) and 0.82 (0.69-0.95) for NSE at 72h; FA vs tNAA/tCr, FA vs NSE and tNAA/tCr vs NSE;  $p = 0.65$ ,  $p = 0.33$  and  $p = 0.55$ , respectively. In the post-hoc analysis, a combination of DTI, 1H-MRS, motor score at 72 hours, epileptic seizures with and without NSE at 72 hours provided the best predictive power with an area under ROC curve of 0.98 (95% CI 0.96 – 1.00) as compared to the next best values of 0.92 (0.86 – 0.97) for a combination of DTI, 1H-MRS and NSE at 72 hours ( $p = 0.009$ ) or to 0.92 (0.85 – 0.98) for a combination of NSE, motor score and epileptic seizures alone ( $p = 0.037$ ) (Table 2).

## Discussion

The main finding of this study was that early stage quantitative DTI or 1H-MRS did not perform better than NSE at 72h for prognosticating poor outcome at six months after OHCA. This was the case also in a subgroup of patients with motor score less than 6 (does not obey commands).

A single measure motor score of  $\leq 3$  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  $\leq 2$  for motor score has 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  $\geq 23$  as compared with current cut-off value of 21 [4, 23]. One explanation may be that in this study the best value of the first 72 hours was used in order to obtain an assessment 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 [24]. Lower fractional anisotropy



values in ischemic white matter probably represent a combination of myelin damage, axonal degeneration, and edema, which all contribute to loss of directional diffusion in white matter tracts [25].

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 [12]. According to earlier evidence most survivors regain consciousness within a week and usually all of them within 10 days after cardiac arrest [4, 5, 26, 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 favorable outcome at six months after cardiac arrest [12]. Current lower prognostic value for fractional anisotropy at early subacute phase can be partly due to evolving white matter injury over time. This suggests that predictive value of fractional anisotropy varies depending on the used imaging time window [29]. Conventional DWI MRI seems to perform better than DTI/MRS in the early phase [30, 31].

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 [10, 12, 32]. 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, without DTI and 1H-MRS, the best prognostic value was provided with a combined model of NSE and motor score at 72h and the presence of epileptic seizures within 72h with AUC of 0.92 as early as 72 hours for all consecutively enrolled OHCA patients with ventricular fibrillation as a primary rhythm. Addition of DTI and 1H-MRS parameters to this model with or without NSE further increased its diagnostic value (AUC 0.98,  $p=0.037$ ).

Although statistically significant, the absolute difference in diagnostic value is still small considering the cost and challenges of MR imaging of critically-ill patients. Therefore early phase DTI/MRS cannot be recommended for clinical practice or at least it should be reserved for very specific cases. However, for research purposes, early phase DTI/MRS can be used as viable surrogate marker for white matter injury. These results warrant further confirmation in another population including also patients with asystole and PEA as primary rhythm.

There are some limitations in this study. First, our results represent a two-center (single country) cohort of patients in successfully resuscitated cardiac arrest victims in whom a shockable rhythm was the initial rhythm at time of resuscitation. Therefore, further validation of the results is required in patients with asystole and pulseless electrical activity. Second, results of pupillary light reflexes at 72 hours were applicable only in 49 patients and were therefore excluded from the final analyses. Third, 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. Fourth, 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. Fifth, 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, early stage DTI or 1H-MRS imaging was not better than NSE at 72h in predicting poor outcome in this patient group. Although the addition of neuroimaging to the best model using clinical and laboratory parameters improved the diagnostic performance, the costs and challenges of MR imaging of critical care patients may outweigh this small benefit. Current results should be prospectively validated in another population of cardiac arrest patients.

## Abbreviations

AUC: Area under the ROC (receiver operating characteristic) curve; CI: Confidence interval; CSI: Chemical Shift Imaging; DTI: Diffusion tensor imaging; DWI: Diffusion weighted imaging; GCS: Glasgow Coma Scale; HR: Hazard ratio; 1H-MRS: Proton magnetic resonance spectroscopy; ICU: intensive care units; IQR: Interquartile range; OHCA: Out-of-hospital cardiac arrest; MRI: Magnetic resonance imaging; mRS: modified Rankin Scale; NSE: Neuron-specific enolase; PPV: Positive predictive value; NPV: Negative predictive value; SD: Standard deviation; ROC: Receiver operating characteristic; ROSC: return of spontaneous circulation; SE-EPI: spin-echo echo planar imaging; TBSS: tract-based spatial statistics; tNAA: Total N-acetyl aspartate; tCho: Total choline; tCr: Total creatine

## Declarations

## Ethical approval and consent to participate

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 Fimea.

## Consent for publication

Not applicable

## Availability of data and materials

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

## Competing interests

TL is a member of the Trial Executive Committee for XePOHCAS, a phase 3 RCT designed to test the efficacy and safety of inhaled xenon in patients with post-cardiac arrest syndrome. 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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## **Authors' 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.

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## Tables

**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
<b>Baseline characteristics</b>				
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)	25 (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
<b>Resuscitation details</b>				
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
<b>Cooling procedure details</b>				
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
<b>Clinical characteristics during ICU stay</b>				
Epileptic seizures, 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
Cumulative dose of norepinehrine within 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.

**Table 2.** Results of magnetic resonance spectroscopy, diffusion tensor imaging, neuron-specific enolase, epileptic seizures, ROSC and motor score in patients with good and poor neurological outcome at six months after OHCA



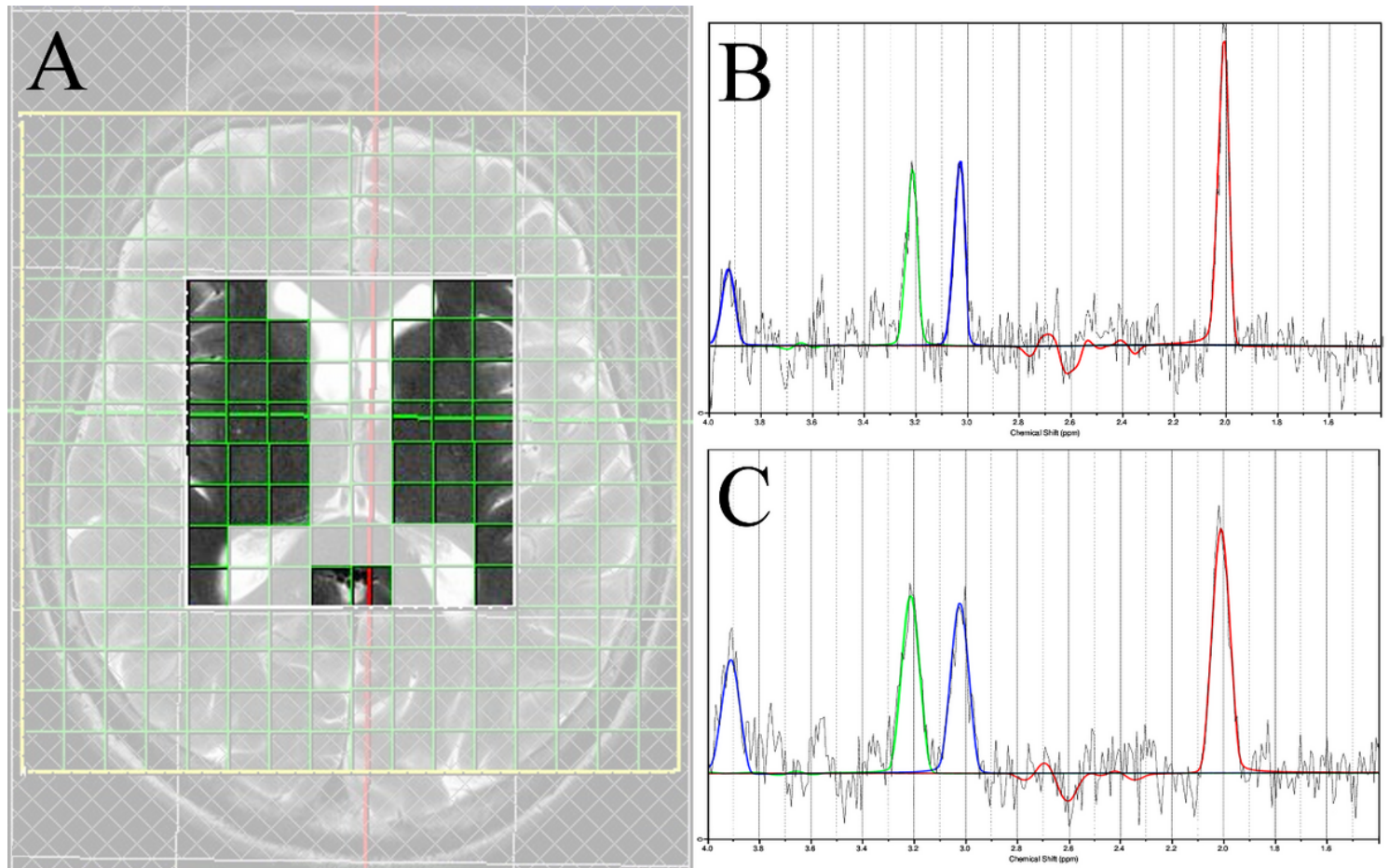
	Unadjusted Mean (SD)		Mean Difference (95% CI)			P-value	
	mRS 0-2	mRS 3-6	Unadjusted	Adjusted <sup>a</sup>		Unadjusted	Adjusted <sup>a</sup>
	(n = 61)	(n = 31)					
tNAA/tCr	2.43 (0.21)	2.18 (0.22)	0.25 (0.15 to 0.34)	0.22 (-0.12 to 0.31)		< 0.0001	< 0.0001
tCho/tCr	0.35 (0.04)	0.35 (0.04)	0.01 (-0.01 to 0.03)	0.01 (-0.01 to 0.02)		0.3831	0.5403
tNAA/tCho	6.96 (0.91)	6.37 (0.68)	0.59 (0.22 to 0.96)	0.54 (0.16 to 0.93)		0.0020	0.0065
Fractional anisotropy	0.43 (0.02)	0.41 (0.03)	0.03 (0.01 to 0.04)	0.02 (0.01 to 0.03)		< 0.0001	0.0014
NSE 48 h <sup>b</sup>	2.97 (0.38)	3.71 (0.71)	-0.73 (-0.96 to -0.51)	-0.74 (-0.98 to -0.49)		< 0.0001	< 0.0001
NSE 72 h <sup>b</sup>	2.71 (0.48)	3.65 (0.87)	-0.94 (-1.22 to -0.66)	-0.91 (-1.21 to -0.61)		< 0.0001	< 0.0001
ROSC, min	20.2 (6.3)	25.3 (6.7)	-5.13 (-7.95 to -2.30)	-5.79 (-8.76 to -2.82)		0.0005	0.0002
			Unadjusted Odds Ratio (95 CI)	Adjusted <sup>a</sup> Odds Ratio (95 CI)			
GCS motor score 1-3 at 72 h, n (%)	9 (14.8)	27 (87.1)	38.99 (10.9 to 138.34)	37.31 (9.85 to 141.32)		< 0.0001	< 0.0001
Epileptic seizures, n (%)	6 (9.8)	19 (61.3)	14.51 (4.78 to 44.05)	19.93 (5.27 to 75.44)		<0.0001	<0.0001
Diagnostic accuracy							
Single parameter	AUC	95% CI	Cut-off	Sensitivity	Specificity	PPV	NPV
tNAA/tCr	0.78	0.68 to 0.88	≤2.313	0.77	0.69	0.56	0.86
tCho/tCr	0.58	0.45 to 0.71	≤0.3445	0.61	0.59	0.43	0.75
tNAA/tCho	0.69	0.58 to 0.80	≤7.154	0.97	0.39	0.45	0.96
Fractional	0.73	0.62	≤0.4235	0.68	0.69	0.53	0.81

anisotropy		to 0.84						
NSE at 48h	0.84	0.74 to 0.94	≥25	0.84	0.79	0.67	0.91	
NSE at 72h <sup>c</sup>	0.85	0.76 to 0.93	≥21	0.77	0.82	0.69	0.88	
ROSC, min	0.72	0.61 to 0.83	≥23	0.71	0.66	0.51	0.82	
GCS motor score at 72h	0.88	0.80 to 0.96	≤3	0.87	0.85	0.75	0.93	
Epileptic seizures	0.76	0.66 to 0.85	yes	0.61	0.90	0.76	0.82	
Combination models	AUC	95% CI	Cut-off	Sensitivity	Specificity	PPV	NPV	
tNAA/tCr and fractional anisotropy	0.83	0.75 to 0.91	NA	0.90	0.59	0.53	0.92	
tNAA/tCr, fractional anisotropy and NSE at 72h	0.92	0.86 to 0.97	NA	0.84	0.85	0.74	0.91	
NSE 72h, GCS motor score at 72h and epileptic seizures	0.92	0.85 to 0.98	NA	0.84	0.93	0.87	0.92	
tNAA/tCr, fractional anisotropy, GCS motor score at 72h and epileptic seizures	0.98	0.96 to 1.00	NA	0.94	0.93	0.88	0.97	
tNAA/tCr, fractional anisotropy, NSE at 72h, GCS motor score at 72h and epileptic seizures	0.98	0.96 to 1.00	NA	0.90	0.95	0.90	0.95	

Values are mean (SD) unless otherwise indicated. <sup>a</sup> Data are adjusted for age, sex, site and treatment group. <sup>b</sup> Values were log-transformed for statistical analysis

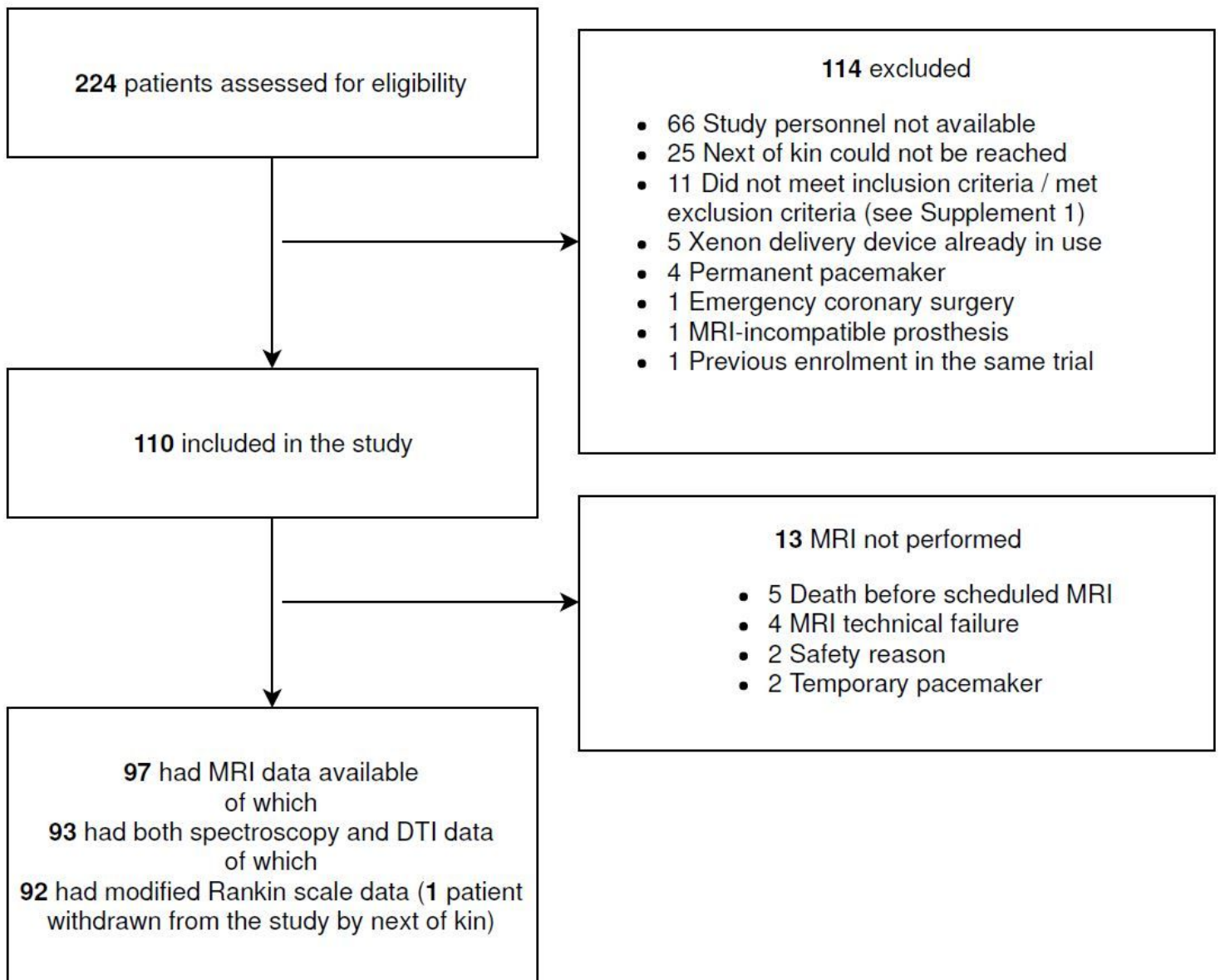
<sup>c</sup> Due to missing data NSE 72h values are applicable for n=60 in mRS 0-2 patients. AUC = the area under the ROC (receiver operating characteristic) curve; CI = confidence intervals; GCS = Glasgow Coma Scale; mRS = modified Rankin Scale; tNAA = total N-acetyl aspartate; NPV = negative predictive value; PPV = positive predictive value; NSE = neuron-specific enolase; ROSC = return of spontaneous circulation; tCho = total choline; tCr = total creatine

## Figures



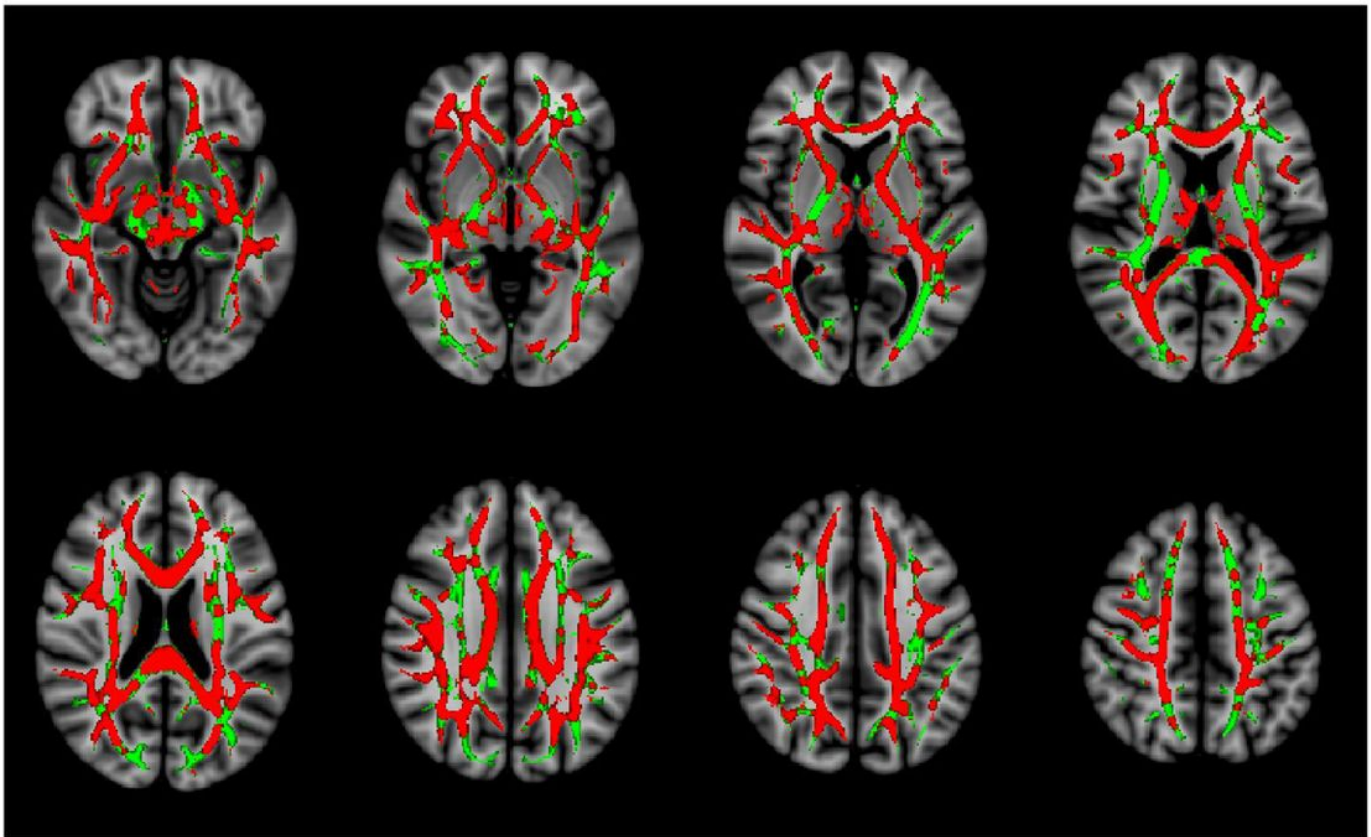
**Figure 1**

1H-MRS analyzed voxels and typical spectra. A) An average of all analyzed voxels in basal ganglia excluding the ones typically including cerebrospinal-fluid was used in the analysis. Voxels outside the analysis area are dimmed. B) A typical spectrum of a single voxel with tNAA/tCr = 2.41 which is close to the mean of the survivors. C) A typical spectrum of a single voxel with tNAA/tCr = 2.19 which is close to the mean of the non-survivors. The signal of total N-acetylaspartate is colored in red, total choline is colored in green and total creatine in blue.



**Figure 2**

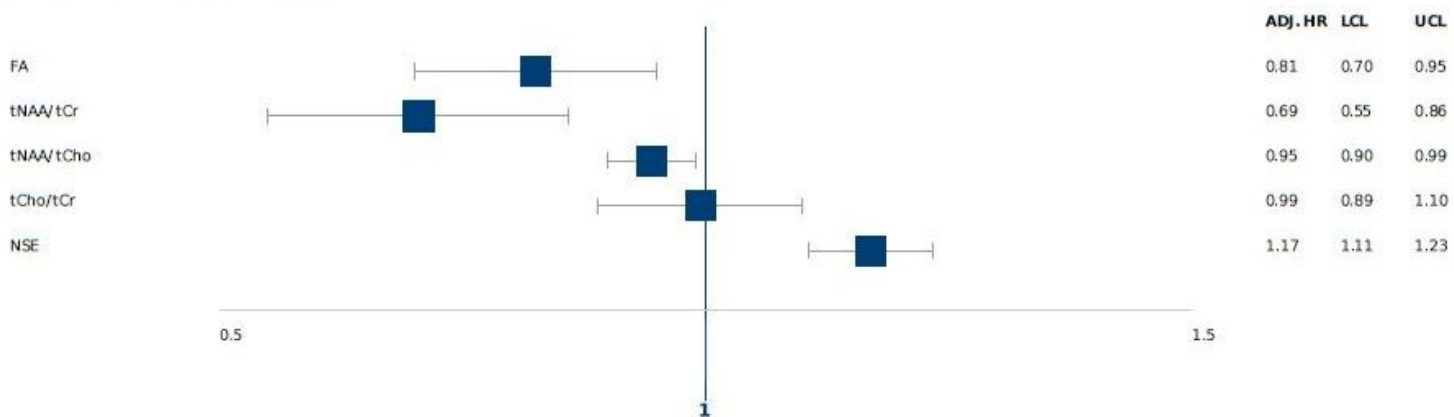
Flow of the participants in the study Comparison of prognostic value of early phase 1H magnetic resonance spectroscopy and diffusion tensor imaging with NSE at 72 hours in comatose survivors of out-of-hospital cardiac arrest – A Sub-study of the Xe-Hypotheca Trial.



**Figure 3**

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., 57.9% 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., 42.1% of all analyzed voxels). According to the Johns Hopkins University white matter tractography atlas [19], 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) (41.6%), cingulum (hippocampal region) (59.6%), forceps minor (74.9%) and major (51.6%), superior longitudinal fasciculus (61.6%), inferior longitudinal fasciculus (57.3%), anterior thalamic radiation (66.0%), inferior fronto-occipital fasciculus (62.5%), corticospinal tract (56.4%), uncinate fasciculus (72.4%), and the body of corpus callosum (87.4%).

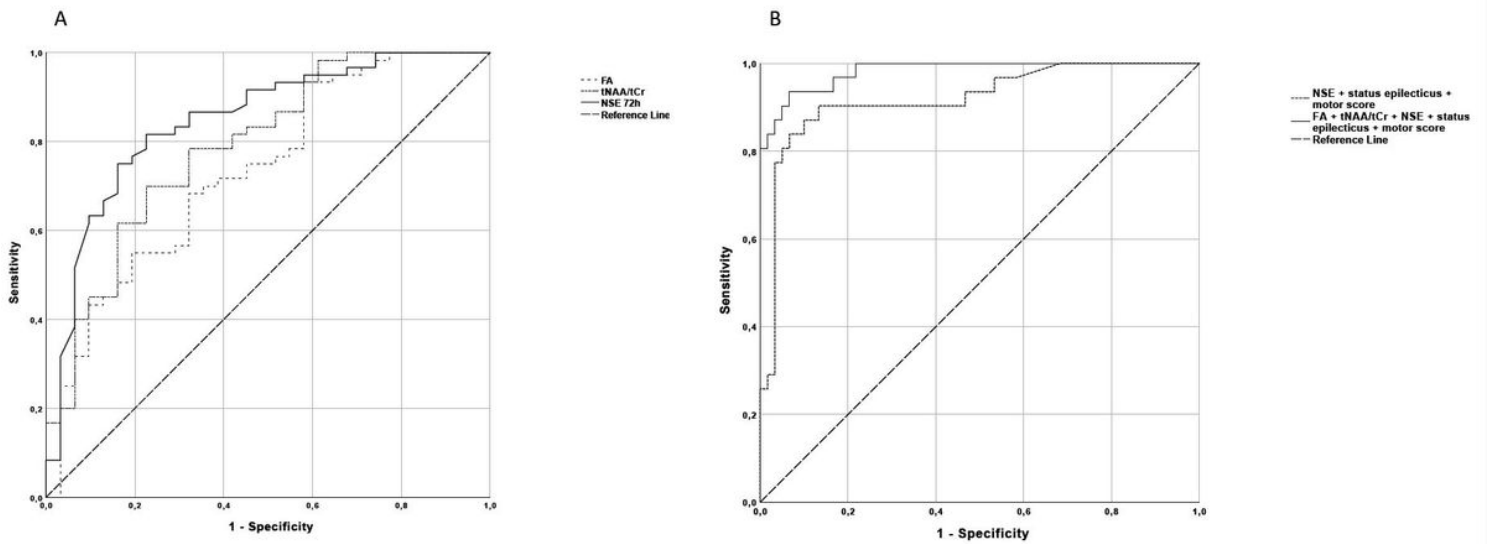
**Adjusted Hazard Ratio for Death**



**Figure 4**

Adjusted Hazard Ratio for Death. The age-, sex-, treatment- and site-adjusted hazard ratios for death in all patients. Hazard ratios are presented for 0.01-unit increase in the global fractional anisotropy, for 0.1-unit increase in tNAA/tCr, for 0.1-unit increase in tNAA/tCho and for 0.01-unit increase in tCho/tCr values, and for 0.1-unit increase in logarithmic scale of NSE.





**Figure 5**

Receiver operating characteristic curves of fractional anisotropy, tNAA/tCr, NSE at 72 hours (A); the combination of NSE, epileptic seizure and motor score at 72 hours and the combination of fractional anisotropy, tNAA/tCr, NSE, epileptic seizure and motor score at 72 hours (B).

## Supplementary Files

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