Supplementary Online Content

Laitio R, Hynninen M, Arola O, et al. Effect of inhaled xenon on cerebral white matter damage in comatose survivors of out-of-hospital cardiac arrest: a randomized clinical trial. *JAMA*. doi:10.1001/jama.2016.1933

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Neurological prognostication after out-of-hospital cardiac arrest in hypothermia treated patients

Neurological prognostication is accomplished if the patient remains unconscious 12 h after rewarming. All sedative medication must have been discontinued 12 h previously, and in hypothermia treated patients prognostication is performed after 72 h post resuscitation.

Neurological examination is performed by consultant neurologist consisting:

1. Glascow Coma Scale (eye, speech and movement responses)
2. Brain stem reflexes
3. Breathing
4. Possible myoclonus

Assessments:

If the patient remains unconscious:

1. Brain CT or MRI scan is performed
2. EEG is obtained to exclude convulsive or nonconvulsive status epilepticus (NCSE). The key considerations:

Continuity of electrical activity of the brain
Response to external stimuli
Spontaneous dynamics in brain electrical activity
Precence of epileptiformic waves
Non-convulsive status epilepticus

Serum neuron specific enolase (NSE) is assessed at 24 h and 48 h ($\pm$2 h) after OHCA

Sensory evoked potential (SEP) assessment is performed if CT/MRI and EEG provide no explanation to unconsciousness

Conclusion

As signs for poor prognosis are:

1. CT/MRI: general cerebral edema with sulcal effacement and wide-spread ischaemia with loss of margins of brain white and grey matter
2. EEG: generalized suppression (<20 μV) or burst suppression, generalized epileptic activity or periodic epileptiformic discharges (PED) with background activity suppression, lack of spontaneous variation and lack of reactivity to external stimuli
3. Continuous refractory to treatments myoclonic status epilepticus with permanent unconsciousness
4. Serum NSE values: ascending trend 24-48 h

5. Unresponsiveness to painful stimuli or extension as the best motor response at 72 h
6. Absent brain stem reflexes at 72 h
7. Bilateral absence of thalamocortical sensory evoked potentials (SEP)
8. Generalized diminished cortical diffusion on MRI

Intensive care treatment protocol

Control and study treatment

The control treatment is MHT. The target core temperature is 33-34 °C. MHT is defined as the time of maintaining target core temperature (TCT), which in this study is 24 hours. MHT of this study is conducted in accordance with national and international recommendations. The current routine MHT protocol of the ICU of Turku University hospital follows the recommendations. Hypothermia is induced with cold intravenous fluids and with endovascular cooling device. Usually, the core temperature has been achieved within 3 hours after arrival to the ICU. Also, most patients are already hypothermic at arrival to the hospital. However, in rare cases, the core temperature has been achieved 8 hours after arrival to the ICU. The target core temperature will be maintained for 24 hours. Thereafter, 0.5 °C / hour rewarming of the patients is allowed to a temperature of 36.5-37.0 °C, which will be maintained until the time of extubation or successful weaning from the respirator. The endovascular catheter is inserted via femoral vein by an experienced anaesthesiologist or intensivist. In endovascular cooling system (e.g. CoolGard 3000^{TM}, Alsius Co), temperature controlled saline circulates within a balloons in a closed loop; the saline never comes in contact with the patient. The cooling of the blood takes place by contact with the balloon membrane (Microtherm^{TM}, Alsius Co). The CoolCard 3000 can cool at a rate between 0.5 – 1.5 °C per hour depending on the endovascular catheter used (Icy^{TM} or Cool Line^{TM} catheter). Core temperature is measured with probes placed in oesophagus and in urinary bladder. After the actual rewarming procedure has been completed, the endovascular catheter will be hold in place until stabilization of the temperature can be assured.

The study treatment is inhaled xenon (LENOXe^{TM}) with subanaesthetic target concentration of 40% in oxygen/air combined with MHT. Details of the MHT are discussed above. The supplier is Air Liquide Deutschland GmbH (Germany). The Finnish National Agency for Medicines (Lääkelaitos) will be provided with the necessary details (including the supplier) of the investigational product before study execution.

Administration of study treatment

A patient can be recruited to this trial if the inclusion criteria, but none of the exclusion criteria, for standard MHT have been fulfilled. A patient will be recruited to this trial after the decision for MHT has already been made by an intensivist or other attending physician. Xenon treatment is initiated in the ICU as soon as possible after the written informed consent has been obtained. Xenon treatment may start before or after target core temperature has been achieved, but it must start within 4 hours after arrival to hospital, or otherwise the patient will be excluded. The xenon treatment will be continued until completion of MHT. The expected duration of xenon inhalation will be approximately 24 hours. Thus, in extreme cases, the duration of xenon inhalation can vary between 18-34 hours depending on the time needed to achieve the core temperature, and on the other hand, to obtain the consent. Xenon will be administered through closed-system ventilator, which is designed for delivery of xenon (PhysioFlex, Physio Dräger, Harlem, The Netherlands).

Target concentration for end-tidal xenon is at least 40%. The concentration is adjusted by flushing the ventilation circuit with extra xenon flushes or nitrogen/air flushes/oxygen. The administration of xenon and the MHT are terminated at the same time (i.e. core temperature ≥ 34 °C). The xenon treatment will be terminated if the core temperature has to be elevated above 34 °C e.g. to treat bradycardia or in the case of early termination of MHT..

A failure to maintain 40% concentration of xenon in oxygen during the treatment is not a reason to remove the study patient from the study. In such case, the treatment will be continued with a possible concentration which can be administered. The end tidal xenon concentration of 65% may not be exceeded nor can it be less than 20% (i.e. the safety limits of xenon concentration).

The end-tidal xenon concentration is electronically monitored, recorded and stored continuously, and it is also recorded on specifically designed Case Report Forms (CRF) in every 30 minutes during the xenon administration.

Prior and concomitant treatments

The patients will be treated according to a normal clinical practise prior and after the study treatment. All concomitant treatments must be recorded on the CRF.

1. Sedation and analgesia

Propofol-infusion (1-5 mg/kg/h) is used as sedative regimen and fentanyl-infusion (50-100 µg/h) and 25- 50 µg i.v. boluses will be administered as an analgesic regimen for all patients while intubated and mechanically ventilated. The dosage of propofol and fentanyl can be increased if needed. Also, remifentanil or oxycodone can be used for analgesia as rescue treatment. Midazolam (bolus of 1-3 mg i.v.) is a first-line rescue treatment for sedative purposes. To prevent shivering and to maintain hypothermia, muscle-relaxant (e.g. cisatracurium) is given as bolus doses and/or by continuous infusion to all patients.

2. Mechanical ventilation

Adjustments of the ventilation parameters i.e. the ventilation mode, tidal volume and frequency are made according to normal treatment of patients in mechanical ventilation. The inspiratory oxygen concentration is adjusted to maintain the partial pressure for arterial oxygen in range of 10-18 kPa. The minute ventilation is adjusted to maintain the partial pressure for arterial carbon dioxide in range of 4.5 – 5.5 kPa. Repeated blood-gas analysis is performed at every hour

during hypothermia and xenon treatment, and later whenever clinically indicated but at least in every 4 hours.

3. Hemodynamics and other

Systemic hypotension (mean arterial pressure < 60 mmHg) is primarily treated with crystalloid fluids or colloids.

Target mean arterial pressure 60-90 mmHg
Systolic arterial pressure ≥ 100 mmHg
Target central venous pressure (PEEP corrected) 6-10 mmHg

Vasopressors (e.g. epinephrine, norepinephrine, dopamine, dobutamine) and antiarrhytmic medication are administered as needed. Maintenance of fluid balance is monitored either by central venous catheter or by fluid intake and output.

Levosimendan (Simdax) can be used.

Hypertension is treated with vasodilators as needed.

Bradycardia treatment during MHT includes boluses of atropine (0.01 mg/kg) and/or a raise of body temperature (0.5 ºC/h) to a degree that is considered necessary. Often in clinical practise, this means a completion of MHT. The final decision to increase body temperature during MHT can be made only by the attending physician. Xenon will be terminated if the core temperature is increased ≥ 34 ºC. A decision to treat bradycardia is always based on clinical grounds, e.g. if general status of the hemodynamics is considered to be inadequate. The final decision can be made by the attending physician only.

Serum glucose will be kept between 4.5 – 6.5 mmol/L and hematocrit between 0.3 and 0.45. Potassium is supplemented if the serum potassium level is < 4 mmol/L. Parenteral nutrition or enteric feeding is initiated as soon as possible. An optimal head position of 30º is provided.

Treatment compliance

Any deviation from the defined protocol of the study treatment must be documented on the CRF.

The end tidal concentrations of xenon will be monitored continuously and recorded electronically on a separate file during the treatment (source data). The concentrations will also be recorded manually on the study treatment CRF. Also, all air or xenon flushes used must be recorded. The absolute consumption of xenon by each patient will be measured afterwards by weighting the xenon cylinders.

Criteria for premature study termination

The independent safety committee and the PI will assemble for a meeting in every 6 months. The committee reserves the right to prematurely terminate the study prior to entry of the intended number of study subjects for valid scientific, ethical or administrative reasons. After such a decision, the investigator shall not enroll more subjects and he must inform the local ethical committee and the National Agency of Medicines of his decision.

The study of individual patient will be terminated by investigator or by the attending physician at any moment during the treatment in the ICU if safety of the patient cannot be assured otherwise. Thereafter, the patient will be treated by the most appropriate means according to the judgment of the attending physician. Each individual case will be considered by the attending physician according to clinical evidence of intensive care medicine. It must be noted that various adverse events occurring during intensive care may not be related to the study treatment and will be considered individually by the attending physician.

The study subjects may be withdrawn from their study treatment prematurely by the investigator or by the physician for one or more of the following reasons:

1. A failure to maintain xenon concentration ≥ 20%

2. A failure of ventilation and/or oxygenation of the patient with the xenon delivery device (Physioflex)
3. If MHT is terminated prematurely; a decision which can be done only by attending physician
4. AE/serious adverse event (SAE)
5. Protocol violation
6. If for any reason the investigator or the attending physician believes that continued participation in the study is not in the best interest of the patient.

A premature termination of the trial will be considered in the case clinically adverse outcome can be shown in the study treatment group after interim analysis (see 10.2). An interim analysis will be performed with 60 patients after they have undergone a 6-months follow-up.

Siemens Magnetom Verio 3T scanner with 12-element Head Matrix coil was used in both MRI centers. Coil configuration allowed the use of Generalized Autocalibrating Partially Parallel Acquisition (GRAPPA) technique to accelerate acquisition (PAT factor of 2 was used). TR time was increased (up to 7900 ms) in DTI sequence, if the number of slices was inadequate to cover whole volume of the brain.

Diffusion tensor imaging processing

Diffusion tensor images were analyzed using TBSS method. Localization and labeling of the tracts were confirmed and identified with JHU white-matter tractography atlas.[1] This method is an observer-independent and hypothesis-free method that provides the ability to spatially locate group differences in DTI data.

Preprocessing of the diffusion tensor imaging data was done using the DTIprep quality control software.[2] The diffusion-weighted images were corrected for the effects of eddy currents and possible motion artifacts. After brain extraction, the diffusion tensor image model was fitted using dtifit tool of the FSL software package. Then, all subjects' diffusion tensor image images were aligned into a common space with affine and diffeomorphic registration using a study-specific template.[3] Diffusion Tensor Imaging ToolKit (DTI-TK, http://www.nitrc.org/projects/dtitk) was used for this step, because its tensor-based registration technique has been shown to be superior to a fractional anisotropy-based registration technique.[4,5] Next, a mean FA image of all subjects' registered FA images was created. This was then skeletonised to create the mean FA skeleton image using the tract-based spatial statistics tools of the FSL toolkit.[3] The skeleton represented the centers of all white matter structures that were

generally common to the subject involved in a study. Each subject's aligned, non-skeletonised FA data were then projected on the mean FA skeleton in such a way that each skeleton voxel takes the FA value from the local center of the nearest relevant tract.[3] These projected FA values were used for voxelwise statistical analysis. Other diffusion metrics (MD, AD, RD) were then projected on the same skeleton and analyzed in similar way.

eTable 1. Inclusion and exclusion criteria

Inclusion Criteria
Witnessed cardiac arrest
Ventricular fibrillation or
Non-perfusing ventricular tachycardia
Presumed cardiac origin
Age 18-80 years
Start of resuscitation by emergency medical personnel within 15 minutes
Return of spontaneous circulation in 45 minutes
Decision for therapeutic hypothermia treatment by attending physician
Exclusion Criteria
Hypothermia (<30 °C core temperature)
Unconsciousness before collapse (cerebral trauma, intoxication etc.)
Computer tomography scan indicating cerebral pathological reason for the cardiac arrest
Responding verbal commands after return of spontaneous circulation
Pregnancy
Coagulopathy
Terminal phase of chronic disease
Systolic arterial pressure less than 80 mmHg lasting >30 minutes after return of spontaneous circulation
Mean arterial pressure less than 60 mmHg lasting >30 minutes after return of spontaneous circulation
Hypoxemia (arterial oxygen saturation <85%) lasting >15 minutes after return of spontaneous circulation
Factors making participation in follow-up implausible
Enrolment in another interventional trial

eTable2. Magnetic resonance imaging protocol

MRI sequence	In-plane resolution (mm)	Slice thickness (mm)	Echo time (ms)	Repetition time (ms)
T2-weighted	0.4 x 0.4	4.0	96	5210
3D FLAIR	1.0 x 1.0	1.0	395	5000
T1-weighted (3D MP-RAGE)	1.0 x 1.0	1.0	2.2	1900
Diffusion tensor imaging[a]	2.0 x 2.0	3.0	100	6100

FLAIR: Fluid Attenuated Inversion Recovery
MP-RAGE: Magnetization Prepared Rapid Gradient Echo
[a]Number of diffusion directions: 20, b-value s/mm^2

eTable 3. Results of the diffusion tensor imaging parameters for quality control from two healthy subjects scanned in both centers

Volunteer 1				
	FA	**MD**	**AD**	**RD**
DTI 20 directions				
scan 1 (Turku)	0.447705	0.000696	0.001056	0.000516
scan 2 (Helsinki)	0.441685	0.000701	0.001062	0.000521
DTI 60 directions				
scan 1 (Turku)	0.380356	0.000544	0.000774	0.000430
scan 2 (Helsinki)	0.385841	0.000547	0.000782	0.000430
Volunteer 2				
	FA	**MD**	**AD**	**RD**
DTI 20 directions				
scan 1 (Turku)	0.414133	0.000751	0.001111	0.000571
scan 2 (Helsinki)	0.424273	0.000746	0.001114	0.000562
DTI 60 directions				
scan 1 (Turku)	0.373503	0.000575	0.000815	0.000456
scan 2 (Helsinki)	0.388167	0.000572	0.000821	0.000447

The scans were performed with the same acquisition parameters and same MRI devices that were used in patients in both centers. However, patients were scanned only with 20 directions in this study. . Essential parameters of DTI sequence with 60 diffusion directions were TR=9100 ms, TE=121 ms, b-value=2500 s/mm2 and voxel size 2.5x2.5x2.5 mm3. Abbreviations: DTI=diffusion tensor imaging; FA=fractional anisotropy; MD=mean diffusivity; AD=axial diffusivity; RD=radial diffusivity

eTable 4. Selected laboratory and treatment parameters

Laboratory parameters between hospital admission and attainment of target temperature	**Xenon (N = 55)**	**Control (N = 55)**	**P value**
Arterial pH	7.3 (7.3-7.4)	7.3 (7.3-7.4)	0.47
Arterial partial pressure for oxygen, kPa	18.1 (14.3-23.5)	17.8 (13.2-24.9)	0.59
Arterial partial pressure for carbon dioxide, kPa	5.2 (4.6-6.0)	5.3 (4.8-5.9)	0.46
Lactate, mmol/l	1.8 (1.2-2.7)	2.1 (1.4-2.8)	0.58
Glucose, mmol/l	8.2 (7.2-10.8)	9.8 (7.3-11.6)	0.1
Hemoglobin, g/l	138 (127-144)	138 (130-146)	0.47
	Xenon (N = 55)	**Control (N = 54[a])**	
Laboratory parameters during hypothermia treatment			
Arterial pH	7.4 (7.4-7.4)	7.4 (7.4-7.4)	0.57
Arterial partial pressure for oxygen, kPa	14.6 (12.3-16.0)	14.4 (13.2-16.5)	0.53
Arterial partial pressure for carbon dioxide, kPa	4.9 (4.6-5.1)	4.7 (4.5-5.1)	0.23
Lactate, mmol/l	1.3 (1.0-1.9)	1.2 (0.9-1.7)	0.2
Glucose, mmol/l	7.0 (6.2-7.8)	7.2 (6.7-7.7)	0.68
Hemoglobin, g/l	128 (120-139)	128 (119-137)	0.72
Treatment Parameters			
Duration of hypothermia treatment, mean hours (95% Confidence intervals)	24.02 (24.01-24.04)	24.02 (23.95-24.10)	0.22
Temperature during hypothermia treatment, ^{o}C	33.1 (33.0-33.3)	33.0 (33.0-33.1)[a]	0.28
Rewarming time, min	636 (440-809)	695 (513-848)[a]	0.12
Time from cardiac arrest to xenon target, min	256 (215-290)		
Mean xenon treatment duration, hours (SD)	24.9 (1.5)		

Values are median (interquartile range) for both groups if not otherwise stated. Values were measured at hospital admission and hourly between intensive care unit admission and initiation of rewarming. An average value per patient was calculated for each assessment [a]One patient in the control group died before

admission to intensive care. Hypothermia treatment was defined as the time temperature was maintained at 33 °C.

eTable 5. Withdrawals of life-sustaining treatment in the intention-to-treat population

	Xenon (N = 55)	**Control (N = 55)**	**P value**
Withdrawals, no.	14	15	0.67
Age, years	66.3 (5.9)	66.5 (6.9)	0.54
Mortality in withdrawals, no.	14	15	0.67
Time from cardiac arrest to withdrawal, days	6.5 (4.0-11)	5.0 (4.0-8.0)	0.43

Values are mean (standard deviation) or median (interquartile range) depending on the distribution of the data. Withdrawal of life-sustaining treatments was carried out based on poor neurology *i.e* either serious hypoxic ischemic encephalopathy and/or drug-resistant status epilepticus in 27 patients. The two remaining patients, one from each group, were withdrawn because of septic multiorgan failure and cardiac failure.

eTable 6. Mode of death

	Xenon (N = 15)	**Control (N = 19)**
Neurological, no (%)	13 (86.7)	16 (84.2)
Cardiological, no (%)	2 (13.3)	2 (10.5)
Multiorgan, no (%)	0 (0.0)	1 (5.3)

Mode of death was defined as previously described.[6]

eTable 7. Diffusion tensor imaging values between survivors and non-survivors for the 6-month follow-up

	Surviving patients (N = 68)	Non-surviving patients (N = 29)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted P value	Adjusted P value
Global values	*Unadjusted Mean (SD)*					
Fractional anisotropy	0.433 (0.026)	0.407 (0.035)	0.77 (0.68 – 0.87)	0.81 (0.69 – 0.94)	<0.0001	0.006
Axial diffusivity, $10^{-3}mm^2/s$	1.193 (0.037)	1.199 (0.076)	1.02 (0.93 – 1.12)	0.98 (0.89 – 1.08)	0.68	0.70
Radial diffusivity, $10^{-3}mm^2/s$	0.598 (0.044)	0.633 (0.076)	1.12 (1.04 – 1.20)	1.07 (0.98 – 1.17)	0.002	0.12
Mean diffusivity, $10^{-3}mm^2/s$	0.796 (0.040)	0.821 (0.074)	1.11 (1.02 – 1.20)	1.05 (0.95 – 1.16)	0.02	0.31

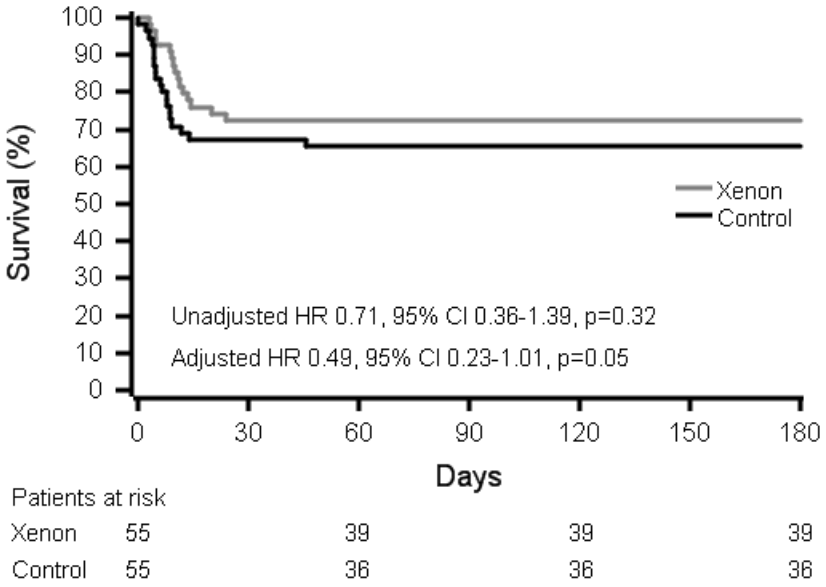
Hazard ratio (HR) was estimated for 0.01 unit increase for the fractional anisotropy and for the diffusivity values. Values were adjusted for age, gender, group and site.

eTable 8. Serious adverse events in the intention-to-treat population

Serious adverse event	**Xenon (N = 48/55)**	**Control (N = 50/55)**	**P value**
Status epilepticus	15	17	0.67
Acute kidney injury			
Risk	14	10	0.36
Injury	2	6	0.27
Failure	2	3	1.00
Pulmonary edema	4	4	1.00
Ventricular fibrillation	6	3	0.49
Ventricular tachycardia	5	8	0.38
Atrial fibrillation	8	4	0.22
Coronary stent thrombosis	1	1	1.00
Sepsis	1	2	1.00
Pneumonia	33	36	0.55
Multi organ failure	1	2	1.00
Adult respiratory distress syndrome	1	1	1.00
Bradycardia treated with pacemaker	1	0	1.00
Third degree atrioventricular block	0	1	1.00
Subarachnoid hemorrhage	1	1	1.00
Carotid dissection	0	1	1.00
Carotid thrombosis	0	1	1.00
Serious bleeding			
Intracranial	0	1	1.00
Gastrointestinal	1	0	1.00
Total	96	102	0.81

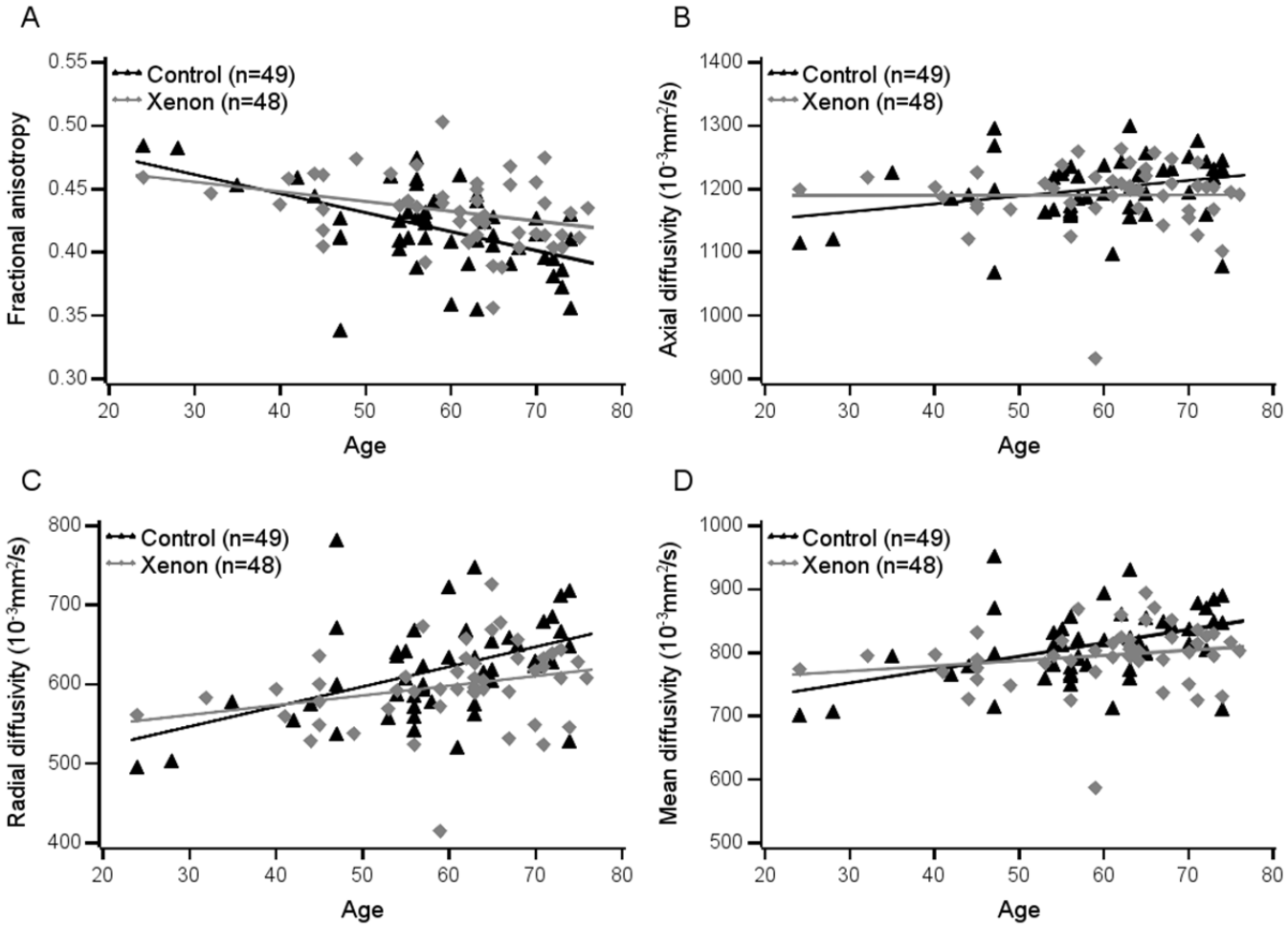
Total number of serious adverse events is presented in the table. Some individuals had more than one adverse event. Serious adverse events include complications during seven days after cardiac arrest as defined by the protocol.

eFigure 1. Probability of survival through the end of the 6-month follow-up in the intention-to-treat population



Shown is Kaplan–Meier estimate of the probability of survivals estimated in the intention-to-treat populations. Values were adjusted with age, gender, time to ROSC, cooling rate and site. The time point 0 indicates the time of out-of-hospital cardiac arrest.

eFigure 2. Results of diffusion tensor imaging as a function of age



Age correlated significantly with fractional anisotropy (r=-0.52, P=0.0001), radial diffusivity (r=0.46, P=0.0009) and mean diffusivity (r=0.42, P=0.003) in the control group and with fractional anisotropy (r=-0.33, P=0.02) in the xenon group. Age did not correlate with axial diffusivity in the control group (r=0.28, P=0.05) or in the xenon group (r=0.00, P=0.99), or with radial diffusivity (r=0.28, P=0.05) and mean diffusivity (r=0.19, P=0.19) in the xenon group. Linear regression lines are shown for the control group and the xenon group.

eReferences

1. Wakana S, Jiang H, Nagae-Poetscher LM, van Zijl PC, Mori S. Fiber tract-based atlas of human white matter anatomy. *Radiology.* 2004; 230(1):77-87.
2. Oguz I, Farzinfar M, Matsui J, et al. DTIPrep: quality control of diffusion-weighted images. *Frontiers in Neuroinformatics*. 2014;8:4. doi:10.3389/fninf.2014.00004.
3. Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. *NeuroImage* 2006;31(4):1487-505.
4. Bach M, Laun FB, Leemans A, et al. Methodological considerations on tract-based spatial statistics (TBSS). *NeuroImage* 2014;15(100):358-69.
5. M. Jenkinson, C.F. Beckmann, T.E. Behrens, M.W. Woolrich, S.M. Smith. FSL. *NeuroImage,* 2012, 62:782-90.
6. Laver S, Farrow C, Turner D, Nolan J. Mode of death after admission to an intensive care unit following cardiac arrest. *Intensive Care Med* 2004;30(11):2126-8.