## Title

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# Effect of Inhaled Xenon on Cerebral White Matter Damage in Comatose Survivors of Out-of-Hospital Cardiac Arrest
## A Randomized Clinical Trial

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**IMPORTANCE** Evidence from preclinical models indicates that xenon gas can prevent the development of cerebral damage after acute global hypoxic-ischemic brain injury but, thus far, these putative neuroprotective properties have not been reported in human studies.

**OBJECTIVE** To determine the effect of inhaled xenon on ischemic white matter damage assessed with magnetic resonance imaging (MRI).

**DESIGN, SETTING, AND PARTICIPANTS** A randomized single-blind phase 2 clinical drug trial conducted between August 2009 and March 2015 at 2 multipurpose intensive care units in Finland. One hundred ten comatose patients (aged 24-76 years) who had experienced out-of-hospital cardiac arrest were randomized.

**INTERVENTIONS** Patients were randomly assigned to receive either inhaled xenon combined with hypothermia (33°C) for 24 hours (n = 55 in the xenon group) or hypothermia treatment alone (n = 55 in the control group).

**MAIN OUTCOMES AND MEASURES** The primary end point was cerebral white matter damage as evaluated by fractional anisotropy from diffusion tensor MRI scheduled to be performed between 36 and 52 hours after cardiac arrest. Secondary end points included neurological outcome assessed using the modified Rankin Scale (score 0 [no symptoms] through 6 [death]) and mortality at 6 months.

**RESULTS** Among the 110 randomized patients (mean age, 61.5 years; 80 men [72.7%]), all completed the study. There were MRI data from 97 patients (88.2%) a median of 53 hours (interquartile range [IQR], 47-64 hours) after cardiac arrest. The mean global fractional anisotropy values were 0.433 (SD, 0.028) in the xenon group and 0.419 (SD, 0.033) in the control group. The age-, sex-, and site-adjusted mean global fractional anisotropy value was 3.8% higher (95% CI, 1.1%-6.4%) in the xenon group (adjusted mean difference, 0.016 [95% CI, 0.005-0.027], $P$ = .006). At 6 months, 75 patients (68.2%) were alive. Secondary end points at 6 months did not reveal statistically significant differences between the groups. In ordinal analysis of the modified Rankin Scale, the median (IQR) value was 1 (1-6) in the xenon group and 1 (0-6) in the control group (median difference, 0 [95% CI, 0-0]; $P$ = .68). The 6-month mortality rate was 27.3% (15/55) in the xenon group and 34.5% (19/55) in the control group (adjusted hazard ratio, 0.49 [95% CI, 0.23-1.01]; $P$ = .053).

**CONCLUSIONS AND RELEVANCE** Among comatose survivors of out-of-hospital cardiac arrest, inhaled xenon combined with hypothermia compared with hypothermia alone resulted in less white matter damage as measured by fractional anisotropy of diffusion tensor MRI. However, there was no statistically significant difference in neurological outcomes or mortality at 6 months. These preliminary findings require further evaluation in an adequately powered clinical trial designed to assess clinical outcomes associated with inhaled xenon among survivors of out-of-hospital cardiac arrest.

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Survivors of out-of-hospital cardiac arrest have a poor prognosis with high rates of death and the likelihood of having severe neurological problems.[1-5] International guidelines recommend the provision of therapeutic hypothermia for comatose survivors of out-of-hospital cardiac arrest; these guidelines are based on 2 studies[2,3] in which patients with an initial shockable rhythm who had been cooled to 33°C achieved a better clinical outcome. However, a recent study[4] suggested that preventing hyperthermia through targeted temperature management to 36°C could be as neuroprotective as 33°C regardless of the initial rhythm at the time of the out-of-hospital cardiac arrest. The optimal temperature for targeted temperature management has yet to be defined and the efficacy of the treatment is modest at best.[2,3]

In animal studies, the neuroprotective properties of the inhaled noble gas xenon have been established; these effects are mediated through multiple putative molecular targets in the pathways involved in postresuscitation brain injury.[6-12] Neuroprotection associated with xenon has been especially evident when combined with hypothermia (33°C to 35°C) and both additive and synergistic interactions have been reported on the basis of histopathological and functional outcomes.[9-12] A recent study[13] demonstrated that xenon inhalation is well tolerated and has favorable cardiovascular properties when administered to patients treated with therapeutic hypothermia after out-of-hospital cardiac arrest.

In this study, we investigated the effect of xenon on the extent of cerebral white matter brain damage as assessed by diffusion tensor magnetic resonance imaging (MRI) in comatose survivors from out-of-hospital cardiac arrest who have hypoxic-ischemic brain injury.

## Methods

### Study Design

We conducted a randomized 2-group single-blind phase 2 clinical drug trial at 2 multipurpose intensive care units 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. An independent data and safety monitoring committee reviewed data after enrollment of every 4 patients and after each 6-month interval.

The study was conducted according to good clinical practice and the current revision of the Declaration of Helsinki[14] guiding clinical drug research in human subjects. Written informed assent was obtained from the next of kin or from the legal representative of the patient within 4 hours after hospital arrival. The patient's family was informed about the right to withdraw from the study at any point but that the data collected until possible withdrawal could be used in the analyses as predefined in the trial protocol (Supplement 1). Patients were informed accordingly if they regained consciousness. The mode of death was classified as neurological, cardiological, or multiorgan as described earlier.[1]

The patients were allocated in 1:1 ratio with random block sizes of 4, 6, and 8 to receive either therapeutic hypothermia treatment alone for 24 hours (defined as the control group) or inhaled xenon (LENOXe, Air Liquide Medical GmbH) in combination with hypothermia for 24 hours (defined as the xenon group). The treatment assignments were randomly generated by a computer. Sequentially numbered sealed envelopes were used separately in the 2 centers for randomization, which was performed after the assent was granted. Clinical investigators performed the patient enrollment, randomization, and intervention assignment. Personnel involved in the treatment of the patient could not be blinded due to practical and safety considerations; however, the MRI analysis of white matter injury was operator-independent, and the neurological end point evaluators as well as the patients were blinded to the treatment.

The diffusion tensor MRI results were not made available to the clinicians treating the patients to avoid bias. For patients in whom there was premature termination of the hypothermia treatment, xenon administration also was discontinued. A local neurological prognostication consensus was used in decisions to withdraw life-sustaining treatment (eMethods in Supplement 2).

### Patients

Comatose survivors of out-of-hospital cardiac arrest consecutively admitted to the Turku and Helsinki University hospitals were screened for eligibility. The main criteria for inclusion were witnessed cardiac arrest from an initial rhythm amenable to defibrillation (ie, ventricular fibrillation or nonperfusing ventricular tachycardia and return of spontaneous circulation within ≤45 minutes). Details of inclusion and exclusion criteria appear in eTable 1 in Supplement 2.

### Treatment Protocol

The patients underwent coronary angiographic interventions when clinically indicated. Integrated care after cardiac arrest and patient monitoring adhered to recent recommendations.[2,15] There was adherence to the detailed treatment protocol regarding cooling treatment and xenon intervention (eMethods in Supplement 2). Inhaled xenon was initiated immediately after randomization through a closed-system ventilator (PhysioFlex, Dräger). Oxygen and air were delivered to achieve an end-tidal xenon concentration of at least 40% (measured continuously by the thermoconductive monitor on the ventilator).

### Outcome

The primary end point was the severity of ischemic white matter brain injury as evaluated by fractional anisotropy from diffusion tensor MRI. Although the study was not powered to detect statistical differences in clinical efficacy, the following variables were collected as exploratory secondary end points: neurological outcome using the Cerebral Performance Category (CPC) score (from 1 [conscious, alert, able to work, might have mild cognitive deficit] to 5 [death]) and the modified Rankin Scale (mRS) score (from 0 [no symptoms] to 6 [death]), mortality at 6 months, and the complication (eg, severe bleeding, pneumonia, sepsis, acute kidney injury,[16] pulmonary edema, ventricular arrhythmias, and epileptic status[17]) rate within 7 days after cardiac arrest.

The Mini-Mental State Examination (MMSE) (score range, 0-30; ≥24 indicates no impairment) and the National Institutes

of Health Stroke Scale (NIHSS) (score range, 0-42; higher scores indicate greater severity) were used to assess the 6-month exploratory neurocognitive outcome. The minimally clinically important differences for CPC and NIHSS have not been established; changes in MMSE of 4 or more points have been interpreted as clinically significant.[18] Differences in mRS score as low as 0.46 have been considered clinically meaningful by practice guidelines.[19,20]

### Diffusion Tensor MRI

The difference in the severity of white matter damage in the control and xenon groups was analyzed by comparing the values of global fractional anisotropy and diffusivity assessment (mean, axial, and radial) obtained from the diffusion tensor MRI. White matter damage evokes 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 indicate more extensive white matter damage.[21] Mean diffusivity values measure overall diffusion and mostly represent cellularity and membrane density; they are affected by several pathological processes (eg, edema and necrosis). Axial diffusivity values represent the amount of water diffusing along the principal axis of the diffusion ellipsoid, whereas radial diffusivity values depict diffusion perpendicular to the principal axis; these values reflect axonal injury and myelin loss, respectively.[22-24]

### MRI Protocol and Image Processing

Patients were scheduled to undergo brain MRI within 16 hours after rewarming; ranging from 36 to 52 hours after out-of-hospital cardiac arrest. Magnetic resonance imaging was performed with a Verio 3T scanner (Siemens Medical Solutions) at both centers, with identical imaging protocols. Diffusion tensor MRI data was preprocessed using DTIprep quality control software (eMethods in Supplement 2). Version 5.0 of the FSL software package (FMRIB) was used for the tract-based spatial statistical analysis.[25-29] This observer-independent and hypothesis-free method has the ability to spatially locate group differences in the diffusion tensor MRI data.

Fractional anisotropy and diffusivity maps were calculated for each patient and these images were projected on a mean skeleton with a voxel size of 1 × 1 × 1 mm (ie, 119 013 voxels per patient), which represents the centers of all major white matter structures. These skeletonized images were then used for voxel-wise statistics to identify all tracts with voxels revealing statistically significant differences in diffusion parameters. Localization and labeling of the major white matter tracts were confirmed according to the Johns Hopkins University white matter tractography atlas.[30] Additional details of the MRI protocol, quality control of imaging, image processing, and tract-based spatial statistics appear in the eMethods, eTable 2, and eTable3 in Supplement 2.

### Statistical Analyses

The sample size was based on a power analysis of fractional anisotropy values. However, a clinically relevant difference in the effect size had not been established. Therefore, this difference was arbitrarily set at 15%, which proved to be unrealistically large given that the difference in the mean fractional anisotropy values between those patients who survived and those who died was only 6.4% (eTable 7 in Supplement 2). The number of patients was predetermined based on the result obtained from the power analysis. Accordingly, it was estimated that 55 patients per group would be needed to reveal an absolute median difference of 15% in fractional anisotropy between the groups (85% power and a 2-sided α level of .05).

The Shapiro-Wilk W test was used to test for normality of all continuous variables. The 2-sample *t* test and the Mann-Whitney test were used to compare continuous variables between the groups. Categorical variables were analyzed with the $\chi^2$ test or the Fisher exact test. The Mann-Whitney test and the Hodges-Lehmann estimate were used for the ordinal variables of mRS and CPC to calculate median differences between the groups. Between-group differences in global fractional anisotropy and diffusivity values (radial, axial, and mean) were analyzed using the 2-sample *t* test and analysis of covariance with adjustment for age, sex, and site as recommended.[31] Permutation-based voxel-wise statistical analysis of fractional anisotropy values was performed with tract-based spatial statistics in conjunction with family-wise error correction for multiple comparisons across space.[29]

The follow-up time for survival analysis was calculated from the time of cardiac arrest until death or 6 months. A complete case analysis was applied to the primary end point due to missing MRI data for 6 patients in the control group and 7 patients in the xenon group. Multiple imputation was not performed due to lack of a good prognostic model to predict valid imputed values for missing data. Kaplan-Meier survival curves and a Cox proportional hazards model were used to compare mortality at 6 months between the groups in the intention-to-treat population. The proportional hazards assumptions were evaluated with the log-cumulative hazard plot and martingale-based residuals; the assumptions were met. 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. Analyses were adjusted with the following prognostic factors: age, sex, time to return of spontaneous circulation, cooling rate, and site.[32] In addition, the global fractional anisotropy and diffusivity values were used as factors associated with 6-month mortality after adjustment for age, sex, group, and site.

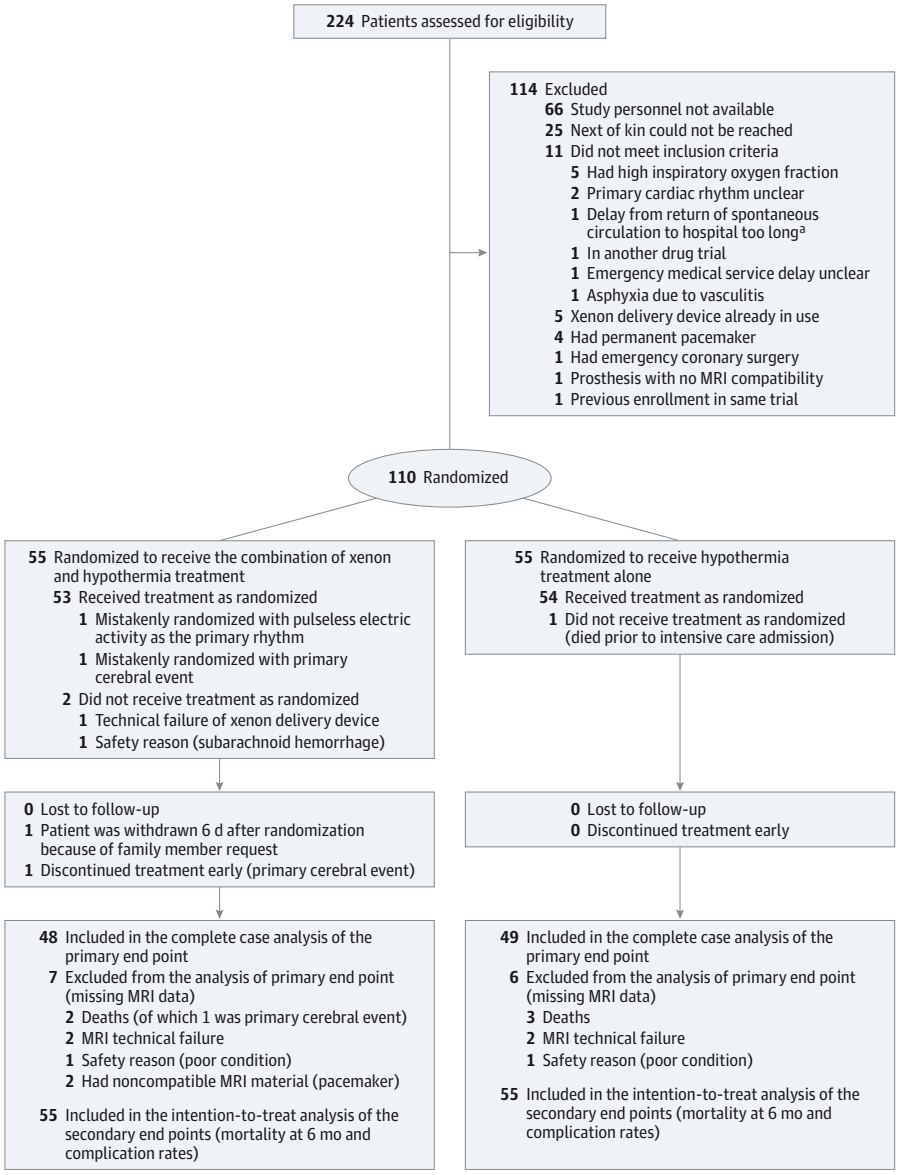
A 2-sided *P* value of less than .05 was considered statistically significant. Secondary end points were not adjusted for multiplicity and were therefore described as exploratory. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc).

## Results

### Patients

Turku University Hospital recruited patients between August 2009 and September 2014; for Helsinki University Hospital, the corresponding dates were between October 2012 and

Figure 1. Flow of Participants in the Study of Effect of Inhaled Xenon on Cerebral White Matter Damage in Comatose Patients Who Experienced Out-of-Hospital Cardiac Arrest




MRI indicates magnetic resonance imaging.

[a] Patient was first transmitted to the wrong hospital; an initiation of possible xenon treatment was not possible within 4 hours.

September 2014; and the 6-month follow-up was completed during March 2015. A total of 224 patients were screened for eligibility and of these, 110 (aged 24 to 76 years) were enrolled with 55 randomly assigned to the xenon group and 55 to the control group (**Figure 1**). The patient characteristics of the 2 randomized groups were similar (**Table 1**). One patient in the xenon group was withdrawn from the study 6 days after the index event by the next of kin.

## Treatment

The mean end-tidal xenon concentration was 48.2% (SD, 3.8%) and the range was 41.4% to 56.9%. Neither core temperatures on admission to the intensive care unit nor during treatment and intervention differed between the groups; time from cardiac arrest to hypothermia target was also similar (Table 1 and eTable 4 in Supplement 2). There were no differences in the routinely measured laboratory parameters (eTable 4). Decisions to withdraw life-sustaining treatment were based on a consensus agreement that there was a poor neurological outcome (eTable 5). Of the 34 patients who died, 29 (85.3%) had a neurological mode of death (eTable 6).

## Primary End Point

Diffusion tensor MRI data for the tract-based spatial statistics analysis were obtained from 48 patients in the xenon group and 49 patients in the control group a median of 53 hours (interquartile range [IQR], 47-64 hours) after cardiac arrest. At 6 months, 75 patients (68.2%) were alive and able to provide data for follow-up. The reasons for missing MRI data appear in Figure 1. Using tract-based spatial statistics analysis, a

Table 1. Baseline Characteristics in the Intention-to-Treat Population[a]

| | Xenon Group (n = 55) | Control Group (n = 55) |
|---|---|---|
| Age, median (IQR), y | 63 (55-70) | 60 (54-67) |
| Male sex | 41 (74.5) | 39 (70.9) |
| Coronary artery disease | 38 (69.1) | 40 (72.7) |
| Hypertension | 22 (40.0) | 26 (47.3) |
| Congestive heart failure[b] | 6 (10.9) | 4 (7.3) |
| Diabetes | 9 (16.4) | 8 (14.5) |
| Asthma or chronic obstructive pulmonary disease | 6 (10.9) | 9 (16.4) |
| Dyslipidemia | 15 (27.3) | 23 (41.8) |
| Smoker | 17 (30.9) | 22 (40.0) |
| ST-elevation myocardial infarction | 15 (27.3) | 19 (34.5) |
| Previous stroke | 10 (18.2) | 12 (21.8) |
| White matter degeneration score | | |
| No. of patients | 48 | 49 |
| Fazekas score 0-1[c] | 39 (81.3) | 38 (77.6) |
| Fazekas score 2-3[c] | 9 (18.8) | 11 (22.4) |
| Resuscitation details | | |
| Bystander resuscitation | 38 (69.1) | 40 (72.7) |
| Delay in emergency medical service, mean (SD), min | 8.4 (3.4) | 8.7 (3.2) |
| Return of spontaneous circulation, mean (SD), min | 22.6 (8.3) | 21.9 (7.0) |
| No flow, median (IQR), min[d] | 0 (0-6) | 0 (0-1) |
| Core temperature before start of cooling, median (IQR), °C | 34.9 (34.4-35.8) | 35.4 (34.0-36.3) |
| Time from out-of-hospital cardiac arrest to target temperature, median (IQR), min | 290 (263-338) | 336 (254-395) |
| Cooling rate, median (IQR), °C/h | 0.43 (0.30-0.50) | 0.43 (0.20-0.50) |
| Time from out-of-hospital cardiac arrest to initiation of xenon, median (IQR), min | 247 (209-278) | |

Abbreviation: IQR, interquartile range.

[a] Data are expressed as No. (%) unless otherwise indicated.

[b] Based on patient history and angiographic findings.

[c] A magnetic resonance imaging system of scoring for white matter hyperintensities that are often observed in older persons (≥65 years) and are considered small vessel disease. A score of 0 indicates no lesions; 1, punctate foci; 2, beginning confluence of foci; and 3, large confluent areas.

[d] Indicates period from cardiac arrest to start of any chest compression by bystander or emergency medical personnel.

statistical parametric map containing 119 013 voxels was created to visualize the spatial distribution of areas with significantly lower fractional anisotropy in the control group (**Figure 2**). The fractional anisotropy was significantly lower in 41.7% of the voxels in the control group than in the xenon group (ie, 58.3% of the voxels did not differ between the groups). The tract-wise distribution of the percentages appears in Figure 2. None of the voxels revealed significantly lower fractional anisotropy values in the xenon group.

The mean global fractional anisotropy values of all voxels presented in Figure 2 were 0.433 (SD, 0.028) in the xenon group and 0.419 (SD, 0.033) in the control group ($P$ = .03; **Table 2**). The age-, sex-, and site-adjusted mean global fractional anisotropy value was 3.8% higher (95% CI, 1.1% to 6.4%) in the xenon group (adjusted mean difference, 0.016 [95% CI, 0.005 to 0.027], $P$ = .006). The mean radial diffusivity value was 0.598 (SD, 0.051) for the xenon group and 0.619 (SD, 0.062) for the control group. The adjusted radial diffusivity value was 3.9% lower (95% CI, 0.5%-7.4%) in the xenon group than in the control group (adjusted mean difference, −0.024 [95% CI, −0.046 to −0.003], $P$ = .03; Table 2). Collectively, these quantitative differences in diffusion tensor MRI values indicated that there had been more severe disruption of white matter in the control group.

Of the diffusion tensor MRI measures, global fractional anisotropy exhibited the best independent predictive value for mortality during the 6-month follow-up period (mean [SD] of 0.433 [0.026] in the surviving patients vs 0.407 [0.035] in those who died per 0.01-unit increase in fractional anisotropy); the age-, sex-, group-, and site-adjusted hazard ratio was 0.81 (95% CI, 0.69-0.94; $P$ = .006) (eTable 7 in Supplement 2). The mean global fractional anisotropy was 6.4% higher (95% CI, 3.3%-9.5%) in the surviving patients than in those who died.

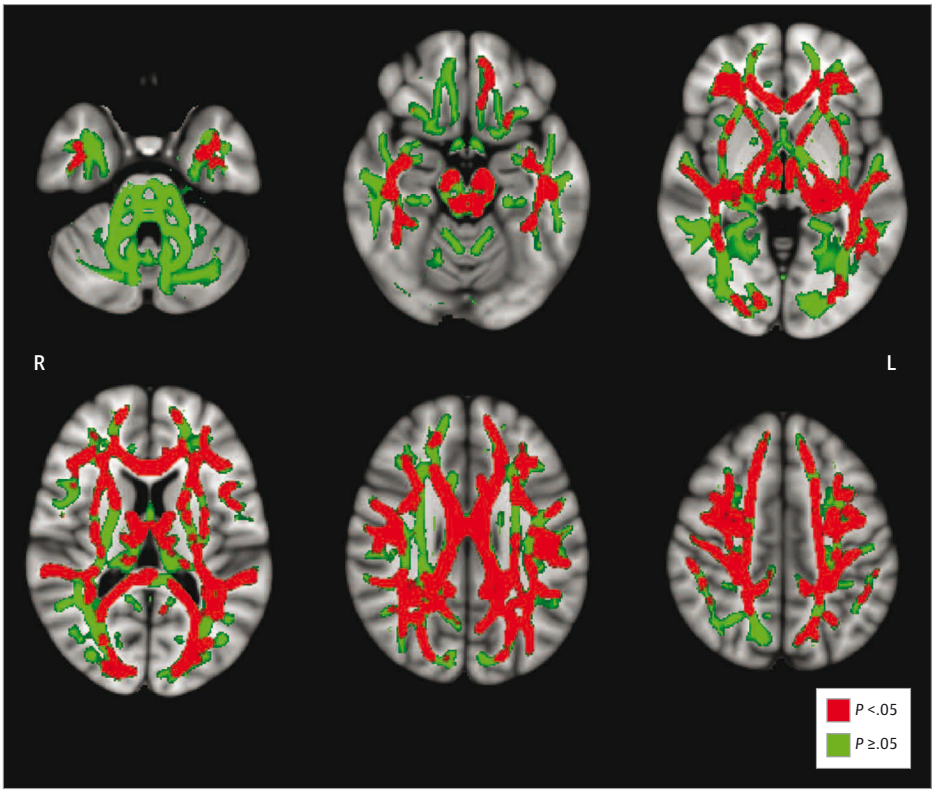
## Secondary End Points

In the intention-to-treat population, the Kaplan-Meier survival estimate after 6-month follow-up was 27.3% (15/55) in the xenon group and 34.5% (19/55) in the control group (adjusted hazard ratio, 0.49 [95% CI, 0.23-1.01], $P$ = .053; eFigure 1 in Supplement 2).

At 6 months, neurological outcome was not significantly different between the groups as assessed with the CPC (median score of 1 [IQR, 1-5] for the xenon group and 1 [IQR, 1-5] for the control group; median difference, 0 [95% CI, 0-0], $P$ = .93) or the mRS (median score of 1 [IQR, 0-6] for the xenon group and 1 [IQR, 0-6] for the control group; median difference, 0 [95% CI, 0-0], $P$ = .68).

Similarly, the rates of serious adverse events were not significantly different between the groups at 6 months (**Table 3** and eTable 8 in Supplement 2). In addition, there was not any difference in neurocognitive outcome measures of MMSE (median of 29 [IQR, 27-30] for the xenon group and median of 29 [IQR, 28-30] for the control group) and NIHSS (median of 0 [IQR, 0-0] for the xenon and control groups).

Figure 2. 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 the xenon group and the control group. Voxels with significantly ($P < .05$, family-wise error corrected for multiple comparisons) lower fractional anisotropy values in the control group were identified and are shown in red in the statistical visualization (ie, 41.7% of all 119 013 analyzed voxels), whereas the areas in which there was no significant difference in fractional anisotropy values between the groups are shown in green (ie, 58.3% of all analyzed voxels). According to the Johns Hopkins University white matter tractography atlas, 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 the control group (marked red in the figure) were as follows: cingulum (cingulate gyrus) (54.6%), cingulum (hippocampal region) (1.4%), forceps minor (49.4%) and major (35.5%), superior longitudinal fasciculus (49.3%), inferior longitudinal fasciculus (34.8%), anterior thalamic radiation (45.1%), inferior fronto-occipital fasciculus (41.0%), corticospinal tract (28.7%), uncinate fasciculus (36.4%), and the body of corpus callosum (72.3%).

Table 2. Results of Diffusion Tensor Magnetic Resonance Imaging in the Complete Case Population

| | Unadjusted Mean (SD) | | Mean Difference (95% CI) | | *P* Value | |
|---|---|---|---|---|---|---|
| Global Values | Xenon Group (n = 48) | Control Group (n = 49) | Unadjusted | Adjusted[a] | Unadjusted | Adjusted[a] |
| Fractional anisotropy | 0.433 (0.028) | 0.419 (0.033) | 0.014 (0.002 to 0.026) | 0.016 (0.005 to 0.027) | .03 | .006 |
| Type of diffusivity, $10^{-3}$ $mm^2/s$ | | | | | | |
| Axial | 1.190 (0.052) | 1.199 (0.051) | −0.009 (−0.030 to 0.012) | −0.011 (−0.031 to 0.010) | .40 | .30 |
| Radial | 0.598 (0.051) | 0.619 (0.062) | −0.021 (−0.043 to 0.002) | −0.024 (−0.046 to −0.003) | .08 | .03 |
| Mean | 0.795 (0.050) | 0.812 (0.056) | −0.017 (−0.038 to 0.005) | −0.020 (−0.040 to 0.0007) | .13 | .06 |

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

## Discussion

The principal finding was that inhaled xenon in combination with therapeutic hypothermia treatment preserved white matter tracts better than hypothermia treatment alone in survivors of cardiac arrest. This was reflected by higher fractional anisotropy values indicative of an attenuation of ongoing disruption of white matter microintegrity, and the lower radial diffusivity, a reflection of less demyelination of the white matter tracts.

Diffusion tensor MRI and tract-based spatial statistics represent an automated quantitative assessment of injury to white matter tracts.[25] The fractional anisotropy, acquired from diffusion tensor MRI, is a correlate of the microintegrity of white matter tracts.[21] Lower fractional anisotropy values obtained within 3 weeks of cardiac arrest have been associated with a poor 1-year

Table 3. Neurological Outcome in the Complete Case Population[a]

| | Description of Category | Xenon Group (n = 54)[b] | Control Group (n = 55) |
|---|---|---|---|
| **Cerebral Performance Category score** | | | |
| 1 | Good cerebral performance: conscious, alert, able to work, might have mild cognitive deficit | 30 (55.6) | 32 (58.2) |
| 2 | Moderate cerebral disability: conscious, sufficient cerebral function for independent daily life | 7 (13.0) | 4 (7.3) |
| 3 | Severe cerebral disability: conscious, dependent on others for daily support; ranges from ambulatory state to severe dementia | 2 (3.7)[c] | 0 |
| 4 | Coma or vegetative state | 0 | 0 |
| 5 | Death | 15 (27.8) | 19 (34.5) |
| **Modified Rankin scale score** | | | |
| 0 | No symptoms | 23 (42.6) | 22 (40.0) |
| 1 | No significant disability: able to carry out all usual activities, despite some symptoms | 7 (13.0) | 8 (14.5) |
| 2 | Slight disability: able to look after own affairs without assistance, but unable to carry out all previous activities | 6 (11.1) | 5 (9.1) |
| 3 | Moderate disability: requires some help, but able to walk unassisted | 1 (1.9) | 1 (1.8) |
| 4 | Moderately severe disability: unable to attend to own bodily needs without assistance | 2 (3.7)[c] | 0 |
| 5 | Severe disability: requires constant nursing care and attention | 0 | 0 |
| 6 | Death | 15 (27.8) | 19 (34.5) |

[a] Data are expressed as No. (%). There was no significant difference between groups in Cerebral Performance Category score ($P$ = .93) or modified Rankin Scale score ($P$ = .68).

[b] One patient was withdrawn from the study 6 days after the index event.

[c] Prior the index event, 1 patient had a Cerebral Performance Category score of 3 (due to mental retardation) and a modified Rankin Scale score of 4.

neurological outcome.[33] We analyzed the discriminating value of the global fractional anisotropy for survival to estimate whether a clinical benefit could accrue from the higher fractional anisotropy values in the xenon group. We found that the mean global fractional anisotropy value was significantly higher among survivors and in the xenon group compared with those who died and those in the control group. In addition, of all the diffusion tensor MRI measures, lower fractional anisotropy was associated with mortality during the 6-month follow-up.

With regard to the other diffusion tensor MRI parameters, each of the diffusivity values was lower in the xenon group; however, the reductions only achieved statistical significance for radial diffusivity (Table 2). In an earlier study in which patients with severe brain injury were scanned at times varying from 5 days to 7 weeks after cardiac arrest, the radial diffusivity values were significantly higher than the corresponding values from healthy volunteers.[34] The mean diffusivity data from 14 patients treated with hypothermia and who received an MRI scan within 3 days of their cardiac arrest did not predict outcome.[35] In this study, higher unadjusted radial and mean diffusivity values were independent predictors of mortality during the 6-month follow-up. However, none of the adjusted diffusivity values could clearly discriminate between survivors and nonsurvivors and hence the clinical value of these metrics remains unproven.

Although the statistically significant difference ($P$ = .006) between patients treated with xenon and those untreated for adjusted mean global fractional anisotropy values was only 3.8% (adjusted mean difference of 0.016), this magnitude of change needs to be considered in the context of the statistically significant difference between survivors and those who died of 6.4% (eTable 7 in Supplement 2). In addition, a diluting effect of voxels, without significant difference between the groups (displayed in green in Figure 2) in the final tract-based spatial statistical analysis, resulted in reduced absolute mean difference in the global fractional anisotropy values (Table 2). Thus, the treatment effect size of xenon on global fractional anisotropy was more than 50% of the difference in this primary outcome measure observed between survivors and those who died. Furthermore, the severity of observed widespread injury was demonstrated by the finding that 41.7% of the white matter tracts on average, including major commissural, associative, and projection fibers, were significantly more severely injured in the control group than in the xenon group. These fibers are involved in multiple important cognitive functions, such as attention, memory, language, emotions, auditory, visual and executive processing, and motor functions of the body.

The volume of white matter constitutes 50% of total brain volume in humans and is highly vulnerable to even brief ischemia.[36] The present results indicate that the higher radial diffusivity (demyelination) was mainly responsible for the lower fractional anisotropy in patients in the control group.[24] An earlier study revealed that N-methyl-D-aspartate receptors are expressed in myelin of white matter oligodendrocytes and become activated during ischemia[37]; xenon is a N-methyl-D-aspartate receptor antagonist and a competitive inhibitor at the glycine coactivation site of that receptor.[7,8] Although not demonstrated here, it is possible that sensitive tests of neurocognitive function in an appropriately powered clinical trial may translate the improvement in white matter into clinical benefits. This hypothesis warrants further investigation.

Although a benefit of xenon on white matter damage was observed, there were no significant differences in the exploratory analysis in either neurological (CPC or mRS score; Table 3) or cognitive outcomes (NHISS or MMSE score) between the 2 treatment groups. The study was underpowered to detect a statistically significant difference in clinical outcome due to the rarity of severe neurological impairment in long-term survivors after cardiac arrest; about 90% of patients who experience cardiac arrest and are alive at the 6-month follow-up have

experienced a good neurological outcome (CPC score of 1 or 2).[38-41] In both groups, the MMSE score was 29 of a maximum score of 30; this was very similar to the score of 28 that was reported in a large targeted temperature management study comparing comatose cardiac arrest survivors treated at 33°C and 36°C, and may reflect the small effect size that this test is capable of eliciting.[40] Therefore, in subsequent studies, more sensitive methods will be needed for assessing functional neurocognitive disorders.

The study had several limitations. First, the secondary end points were not adjusted for multiplicity and therefore were described as exploratory. Second, in the analysis of secondary end points, we did not have sensitive enough neurocognitive tests to measure subtle degrees of functional disability in the small number of patients that could be assessed. Third, our primary end point was based on a single time point in the early stage of evolving brain injury; it remains to be determined whether the improvement in the diffusion tensor MRI metrics persists and can contribute to a long-term clinical benefit. Fourth, all patients received delayed xenon intervention and therefore it is possible that the observed effect on white matter may have been different if the xenon intervention had been initiated earlier after the ischemic event. Fifth, although more than 80% of the deaths in this study were due to severe ischemic brain injury, underlying cardiac disease also may have contributed to poor outcomes, therefore, it is unknown whether some patients might have benefitted from the improved cardiovascular stability associated with xenon.[13] Sixth, the sample size calculation of the present study was based on an unrealistic assumption of the absolute difference of fractional anisotropy values between the groups. Nonetheless, because the variance of the variable was proportionally smaller, there was a statistically significant difference between the groups; however, the absolute difference in fractional anisotropy was small. Seventh, the predictive value of global fractional anisotropy for survival needs to be prospectively validated before designating the improvement in white matter injury seen in the xenon group as a marker for improved survival.

## Conclusions

Among comatose survivors of out-of-hospital cardiac arrest, inhaled xenon combined with hypothermia compared with hypothermia alone resulted in less white matter damage as measured by fractional anisotropy of diffusion tensor MRI. However, there was no statistically significant difference in neurological outcomes or mortality at 6 months. These preliminary findings require further evaluation in an adequately powered clinical trial designed to assess clinical outcomes associated with inhaled xenon among survivors of out-of-hospital cardiac arrest.

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## REFERENCES

1. 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):2126-2128.

2. Neumar RW, Nolan JP, Adrie C, et al. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication. A consensus statement from the International Liaison Committee on Resuscitation; the American Heart Association Emergency Cardiovascular Care Committee; the Council on Cardiovascular Surgery and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the Council on Clinical Cardiology; and the Stroke Council. *Circulation*. 2008;118(23):2452-2483.

3. Hypothermia after Cardiac Arrest Study Group. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. *N Engl J Med*. 2002;346(8):549-556.

4. Nielsen N, Wetterslev J, Cronberg T, et al; TTM Trial Investigators. Targeted temperature management at 33°C versus 36°C after cardiac arrest. *N Engl J Med*. 2013;369(23):2197-2206.

5. Zeiner A, Holzer M, Sterz F, et al. Hyperthermia after cardiac arrest is associated with an unfavorable neurologic outcome. *Arch Intern Med*. 2001;161(16):2007-2012.

6. Wilhelm S, Ma D, Maze M, Franks NP. Effects of xenon on in vitro and in vivo models of neuronal injury. *Anesthesiology*. 2002;96(6):1485-1491.

7. Franks NP, Dickinson R, de Sousa SL, Hall AC, Lieb WR. How does xenon produce anaesthesia? *Nature*. 1998;396(6709):324.

8. Banks P, Franks NP, Dickinson R. Competitive inhibition at the glycine site of the N-methyl-D-aspartate receptor mediates xenon neuroprotection against hypoxia-ischemia. *Anesthesiology*. 2010;112(3):614-622.

9. Thoresen M, Hobbs CE, Wood T, Chakkarapani E, Dingley J. Cooling combined with immediate or delayed xenon inhalation provides equivalent long-term neuroprotection after neonatal hypoxia-ischemia. *J Cereb Blood Flow Metab*. 2009; 29(4):707-714.

10. Chakkarapani E, Dingley J, Liu X, et al. Xenon enhances hypothermic neuroprotection in asphyxiated newborn pigs. *Ann Neurol*. 2010;68 (3):330-341.

11. Hobbs C, Thoresen M, Tucker A, Aquilina K, Chakkarapani E, Dingley J. Xenon and hypothermia combine additively, offering long-term functional and histopathologic neuroprotection after neonatal hypoxia/ischemia. *Stroke*. 2008;39(4):1307-1313.

12. Ma D, Hossain M, Chow A, et al. Xenon and hypothermia combine to provide neuroprotection from neonatal asphyxia. *Ann Neurol*. 2005;58(2): 182-193.

13. Arola OJ, Laitio RM, Roine RO, et al. Feasibility and cardiac safety of inhaled xenon in combination with therapeutic hypothermia following out-of-hospital cardiac arrest. *Crit Care Med*. 2013; 41(9):2116-2124.

14. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191-2194.

15. Langhelle A, Nolan J, Herlitz J, et al. Recommended guidelines for reviewing, reporting, and conducting research on post-resuscitation care. *Resuscitation*. 2005;66(3):271-283.

16. Chua HR, Glassford N, Bellomo R. Acute kidney injury after cardiac arrest. *Resuscitation*. 2012;83 (6):721-727.

17. Brophy GM, Bell R, Claassen J, et al. Guidelines for the evaluation and management of status epilepticus. *Neurocrit Care*. 2012;17(1):3-23.

18. Burback D, Molnar FJ, St John P, Man-Son-Hing M. Key methodological features of randomized controlled trials of Alzheimer's disease therapy. Minimal clinically important difference, sample size and trial duration. *Dement Geriatr Cogn Disord*. 1999;10(6):534-540.

19. Chaisinanunkul N, Adeoye O, Lewis RJ, et al. Adopting a patient-centered approach to primary outcome analysis of acute stroke trials using a utility-weighted modified Rankin scale. *Stroke*. 2015;46(8):2238-2243.

20. Berkhemer OA, Fransen PSS, Beumer D, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med*. 2015;372(1): 11-20.

21. Le Bihan D. Looking into the functional architecture of the brain with diffusion MRI. *Nat Rev Neurosci*. 2003;4(6):469-480.

22. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *Neuroimage*. 2002;17(3): 1429-1436.

23. Song SK, Sun SW, Ju WK, Lin SJ, Cross AH, Neufeld AH. Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. *Neuroimage*. 2003;20(3):1714-1722.

24. Song SK, Yoshino J, Le TQ, et al. Demyelination increases radial diffusivity in corpus callosum of mouse brain. *Neuroimage*. 2005;26(1):132-140.

25. Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage*. 2006;31 (4):1487-1505.

26. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage*. 2004;23(1)(suppl 1):S208-S219.

27. Woolrich MW, Jbabdi S, Patenaude B, et al. Bayesian analysis of neuroimaging data in FSL. *Neuroimage*. 2009;45(1)(suppl):S173-S186.

28. Mori S, Oishi K, Jiang H, et al. Stereotaxic white matter atlas based on diffusion tensor imaging in an ICBM template. *Neuroimage*. 2008;40(2):570-582.

29. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage*. 2009;44(1):83-98.

30. 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.

31. Hsu JL, Leemans A, Bai CH, et al. Gender differences and age-related white matter changes of the human brain. *Neuroimage*. 2008;39(2): 566-577.

32. Lin S, Scales DC, Dorian P, et al. Targeted temperature management processes and outcomes after out-of-hospital cardiac arrest. *Crit Care Med*. 2014;42(12):2565-2574.

33. Luyt CE, Galanaud D, Perlbarg V, et al. Diffusion tensor imaging to predict long-term outcome after cardiac arrest. *Anesthesiology*. 2012;117(6):1311-1321.

34. van der Eerden AW, Khalilzadeh O, Perlbarg V, et al. White matter changes in comatose survivors of anoxic ischemic encephalopathy and traumatic brain injury. *Radiology*. 2014;270(2):506-516.

35. Wu O, Sorensen AG, Benner T, Singhal AB, Furie KL, Greer DM. Comatose patients with cardiac arrest: predicting clinical outcome with diffusion-weighted MR imaging. *Radiology*. 2009; 252(1):173-181.

36. Pantoni L, Garcia JH, Gutierrez JA. Cerebral white matter is highly vulnerable to ischemia. *Stroke*. 1996;27(9):1641-1646.

37. Káradóttir R, Cavelier P, Bergersen LH, Attwell D. NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. *Nature*. 2005;438(7071):1162-1166.

38. Stiell IG, Nesbitt LP, Nichol G, et al. Comparison of the Cerebral Performance Category score and the Health Utilities Index for survivors of cardiac arrest. *Ann Emerg Med*. 2009;53(2):241-248.

39. Lilja G, Nielsen N, Friberg H, et al. Cognitive function in survivors of out-of-hospital cardiac arrest after target temperature management at 33°C versus 36°C. *Circulation*. 2015;131(15):1340-1349.

40. Cronberg T, Lilja G, Horn J, et al. Neurologic function and health-related quality of life in patients following targeted temperature management at 33°C vs 36°C after out-of-hospital cardiac arrest. *JAMA Neurol*. 2015;72(6):634-641.

41. Nielsen N, Hovdenes J, Nilsson F, et al. Outcome, timing and adverse events in therapeutic hypothermia after out-of-hospital cardiac arrest. *Acta Anaesthesiol Scand*. 2009;53(7):926-934.