Clinical Study Protocol

DEXmedetomidine to Improve Neurologic Injury of patients after out-of-hospital cardiac arrest – a phase II randomized, double blind, placebo-controlled, parallel group trial

EU-CT trial number: 2026-527007-21-00

Study short title

The DEX-INI trial

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1 SPONSOR, INVESTIGATOR, MONITOR AND SIGNATURES

Sponsor/or representative (OEL)

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Role of the Funding Source:

This study is funded monetarily by the Austrian Science Funds. This study was submitted as an investigator-initiated study and the funding source had no influence on any aspects of the trial design. The funder has no direct involvement in the trial.

Role of the Sponsor:

This is an academic trial that is sponsored by the Medical University of Vienna with Assoz.Prof.Priv.Doz.Dr. Markus Zeitlinger being the representative of the sponsor. The sponsor has the right to review and comment on the protocol.

Role of the Investigator:

Trial design, conduct of the trial and data analysis are the responsibility of the Investigator.

Trial registration:

This trial is registered at the Clinical Trial Information System with the EU-CT trial Number 2026-527007-21-00. This trial is registered at the ISRCTN registry.

Protocol and statistical analysis plan:

The trial protocol including the statistical analysis plan will be made available at the above mentioned-registries.

Data Sharing:

De-identified individual data will be shared with qualified researchers upon reasonable request (submission of a methodologically sound proposal) to the investigator. The sponsor representative must also agree to sharing the data. Only data necessary to complete the respective scientific question will be shared. Data will be made available via a secure portal beginning 12 months after publication of the trial results.

Conflicts of Interest:

The investigators report no relevant conflict of interest.

Dissemination policy

The study results will be published in peer-reviewed journals and may be presented at international scientific meetings, in accordance with the policies of the Austrian Science Funds.

2 PROTOCOL SYNOPSIS

SCIENTIFIC TITLE	DEXmedetomidine to Improve Neurologic Injury of patients after out-of-hospital cardiac arrest – a phase II randomized, double blind, placebo-controlled, parallel group trial
PUBLIC TITLE	Testing if dexmedetomidine, a sedative drug, helps to protect the brain after sudden cardiac arrest outside a hospital
OBJECTIVES	**Primary Objective** • To investigate the effects of dexmedetomidine in patients after out-of-hospital cardiac arrest in comparison to placebo, as measured by the ranked composite of death before 72h and of neuron-specific enolase (NSE) 72h after cardiac arrest **Secondary Objectives** • To investigate the effects of dexmedetomidine in patients after out-of hospital cardiac arrest in comparison to placebo, as measured by concentrations of neuron-specific enolase 72h after cardiac arrest and during the first 72h after cardiac arrest • To investigate the effects of dexmedetomidine in patients after out-of-hospital cardiac arrest in comparison to placebo, as measured by the ranked composite of death before 72h and of S100ß 72h after cardiac arrest • To investigate the effects of dexmedetomidine in patients after out-of-hospital cardiac arrest in comparison to placebo, as measured by concentrations of S100ß 72h after cardiac arrest and during the first 72h after cardiac arrest • To assess the effects of dexmedetomidine on the occurrence of acute kidney injury or hypoxic liver injury occurring up to 72h after cardiac arrest • To assess the effect of dexmedetomidine on the extent of cardiac injury in patients with acute coronary syndromes as causative disease (i.e., those who require coronary interventions) up to 72h after cardiac arrest • To assess the effects of dexmedetomidine on exploratory biomarkers up to 72h after cardiac arrest • To investigate the underlying mechanisms of dexmedetomidine on endothelial barrier function by performing in vitro experiments using endothelial cell cultures • To investigate the pharmacokinetics of dexmedetomidine in patients undergoing target temperature management at 33° Celsius
DESIGN / PHASE	Prospective, single-center, randomized, parallel group, double-blind, placebo controlled, phase II study.

STUDY PLANNED DURATION	**First patient** **First visit**	*4Q* 2026	**Last patient** **First visit**	*4Q* 2028	**Last patient** **Last visit**	*2Q* *2029*
CENTER(S) / COUNTRY(IES)	1 center in 1 country. Austria					
PATIENTS / GROUPS	120 patients after out-of-hospital cardiac arrest 60 patients per group (dexmedetomidine vs. placebo) Randomization ratio 1:1, stratification based on time to Return of Onset of Spontaneous Circulation (ROSC) (≤30 minutes and >30 minutes) and necessity of mechanical circulatory support (MCS)					
INCLUSION CRITERIA	• Adult patients ≥18 years of age experiencing out-of-hospital cardiac arrest • Sustained circulation (defined as ROSC or successful eCPR flow lasting for at least 20 minutes) within 90 minutes of suspected collapse • Comatose (Glasgow Coma Scale <8) on admission or suspected in patients receiving sedatives • Start of the trial drug is possible within 2 hours of hospital admission • Subjected to temperature control • Combined no-flow and low-flow time of ≥10 minutes					
EXCLUSION CRITERIA	• Expected death within 72 hours or known medical condition limiting expected survival to < 3 months • Known limitations to intensive care therapy and “Do Not Resuscitate” or “Allow Natural Death” orders • Advanced directive of patient to decline resuscitation, intensive care measures, or participation in a clinical trial according to the local legal requirements of participating trial sites • Known pre-arrest CPC of 3 or 4 • Known diseases that may interfere with biomarkers indicative of neurologic injury (e.g., cancer, inflammation, acute bleeding, stroke or infection of the central nervous system) at the discretion of the investigator • Time to sustained circulation > 90 minutes • No-flow time >10 minutes • Unwitnessed cardiac arrest unless the no-flow time can safely be assumed to be <10minutes • Unmanageable hemodynamic and respiratory instability albeit the use of vasopressors and/or mechanical circulatory support – at the discretion of the treating physicians • Known allergies or intolerances against any of the substances used in the trial • Requirement of acute surgery at screening • Pregnancy or breastfeeding					
STUDY PERIODS	• Screening immediately after admission (treatment start within 2h of admission must be possible)					

	• Treatment period: 24h • Observation period: 72h • Follow-ups: Day 30 and Day 90
INVESTIGATIONAL DRUG	**Dexmedetomidine:** initial dose: 0.5µg/kg/h maintenance dose: 0.5µg/kg/h **Placebo:** 0.9% sodium chloride
AUXILIARY MEDICINAL PRODUCT	Not applicable
CONCOMITANT MEDICATION	**Allowed** Standard treatment after out-of-hospital cardiac arrest is allowed **Not allowed** Concomitant treatment with dexmedetomidine.
EFFICACY ENDPOINTS	Biomarkers indicative of neurologic injury (neuron specific enolase, S100ß) Biomarkers indicative of inflammatory response (e.g., C-reactive protein, interleukin-6) Frequency of acute renal injury and hypoxic hepatic injury Biomarkers indicative of cardiac injury in patients with an acute coronary syndrome as causative disease Cerebral Performance Categories (CPC) on day 30 and 90 Modified Rankin Scale (mRs) on day 30 and 90 Mortality on day 30 and 90 Exploratory biomarkers Quality of Life markers (see below)
TOLERABILITY / SAFETY ENDPOINTS	Adverse events Serious adverse events Adverse events of specific interest (e.g., bradycardia requiring treatment, hemodynamic instability requiring dose reduction or interruption of treatment)
PHARMACOKINETIC / PHARMACODYNAMIC ENDPOINTS	Pharmacokinetic parameters determined by non-compartmental pharmacokinetic analysis and population pharmacokinetic modelling and simulation (e.g., clearance, volume of distribution, half-life)
EXPLORATORY ENDPOINTS	Endothelial Barrier Function using cell cultures and patient plasma Exploratory biomarkers

QUALITY OF LIFE / PHARMACOECONOMIC ENDPOINTS	EuroQol Health related Questionnaire index (EQ-5D-5L) and EQ visual analogue scale Hospital Anxiety and Depression Scale (HADS) Short Form-36 Barthel Index
STATISTICAL METHODOLOGY	**Primary Endpoint** Ranked composite of death before 72 h and NSE concentration at 72 h between the dexmedetomidine and the placebo period. **Null and alternative hypotheses:** *H_0 There is no difference in the ranked composite endpoint of neuron-specific enolase 72h and death before 72h after cardiac arrest between the two study groups* *H_1: There is a difference in the ranked composite endpoint of neuron-specific enolase 72h and death before 72h after cardiac arrest between the two study groups* Type-I and -II errors - power. *Type I error: 5%, two-sided Type II error: 15% Power: 85%* **Sample size calculation** In this trial, we will investigate the effects of dexmedetomidine on NSE values at 72 hours after cardiac arrest. There are no data on the expected effects of dexmedetomidine on clinical outcomes after OHCA or on biomarkers of neurologic injury in humans. Although many publications address the performance of NSE as a biomarker for neurologic outcome, only a few report NSE as an outcome parameter in prospective, controlled trials with similar inclusion and exclusion criteria. In a study by Nutma et al., using similar inclusion and exclusion criteria, NSE concentrations were 56 ± 13 μg/L (mean ± standard deviation [SD]) at 24 hours and 52 ± 14 μg/L at 72 hours after ROSC in the control group. These values are supported by data from a double-blind, randomized, placebo-controlled trial in swine, in which NSE concentrations in the placebo group reached 45 ± 8 μg/L after 24 hours, while they were 30 ± 8 μg/L in the low-dose group, which received 0.25 μg/kg/h dexmedetomidine, and 25 ± 5 μg/L in the high-dose group, which received 0.5 μg/kg/h. In addition, we have analyzed unpublished data from the Vienna Cardiac Arrest Registry from 2019 to 2024. A total of 1204 cardiac arrest cases were treated at the Department of Emergency Medicine during that

	period. We aimed to use selection criteria that would allow analysis of a somewhat similar population (e.g., out-of-hospital cardiac arrest, time to ROSC of 10-60 minutes, cardiac and pulmonary cause of cardiac arrest, etc.). Of the remaining 268 cases, NSE values at 72h were available for only 116 (43%) patients. In the majority of the 152 missing 72h NSE values, the patient did not survive to 72h (n=86, 57%), making it impossible to measure NSE values. For the remaining missing values, one possible explanation is that the 48h values were very low, the patients' clinical course was favorable, and omitting NSE measurements was a deliberate choice. Another reason may be very high values at 48h, which also rendered the 72h value not very useful. To increase the sample size, we chose to impute NSE values at 48h when 72h values are unavailable, resulting in 160 (60%) patients. These assumptions yield a mean NSE of 58 ± 51 µg/L. The higher variability is not surprising, given that these patients were not included in a clinical trial with defined in- and exclusion criteria, but were collected consecutively. Furthermore, part of the variability may also be explained by data imputation. Because the trial will apply strict inclusion criteria and standardized sampling, we assumed a lower SD. For the sample size calculation, we aimed to take these considerations into account and assumed a mean NSE concentration of 58 ± 40 µg/L in the control group and 38 ± 28 µg/L in the dexmedetomidine group 72 hours after ROSC. This corresponds to a 33% reduction in NSE concentrations, which is similar to the study in swine and to other clinical situations: e.g., in patients undergoing epilepsy surgery, dexmedetomidine reduced NSE after 24h to 13.5 ± 9.1 µg/L compared with 32.8 ± 43.4 µg/L in the control group. We have further assumed a two-sided alpha level of 5% and 80% power. This would result in a sample size of n=51 per group (calculated with a Wilcoxon- Mann-Whitney-U test using G.Power 3.1). Given the uncertainty regarding the expected treatment effect, the high variability of NSE concentrations observed in institutional registry data, and possible missing data, we increased the target power to 85%. This resulted in a required sample size of 58 patients per group, which was rounded to 60 patients per group. **Statistical methodology** Interim analysis Not applicable Main analysis set(s) Modified Intention to treat population (= all study participants in whom the intervention was started)

	Sensitivity analysis set(s) Per protocol population (= all study participants in whom the intervention was administered as planned in the protocol without interruption, dose reduction or termination) Baseline data and demographics will be analyzed using descriptive statistics. Continuous variables will be summarized using mean and standard deviation or median and interquartile ranges (IQR), and 5th and 95th percentiles, as applicable. Categorical variables will be summarized as counts and percentages. All statistical tests will use a two-sided alpha error of 5%. The primary endpoint will be a ranked composite of death before 72 hours after ROSC and NSE concentration at 72 hours. Patients who die before 72 hours will be assigned the worst outcome rank, below all observed 72-hour NSE values among survivors. Among patients alive at 72 hours with available measurements, lower NSE concentrations will be considered more favorable and will be ranked accordingly. The sample size was estimated based on a clinically meaningful difference in NSE concentrations at 72 hours between treatment groups, informed by data from comparable populations. Assuming a 33% relative reduction in NSE concentrations at 72 hours in the dexmedetomidine group as compared with the placebo group, a two-sided alpha level of 0.05, and a power of 85%, the required sample size was 60 patients per group, calculated using a Mann-Whitney-U test for two independent groups. The primary analysis will compare the distribution of the ranked endpoint between the dexmedetomidine and placebo groups using a Wilcoxon-Mann-Whitney test. Effect estimates will be expressed as the probability that a randomly selected patient in the dexmedetomidine group has a more favorable outcome than a randomly selected patient in the placebo group, with corresponding confidence intervals. Values >0.5 favor dexmedetomidine. Although, missing data should be minimal, given that (i) all patients will be hospitalized, (ii) the active period of the trial is short, and (iii) we will assume highest ranks for those patients who die within the first three days of admission, we will conduct a sensitivity analysis in which 48h values will be substituted for 72h (last observation carried forward) in case of missing values. As a supportive analysis NSE concentrations and all longitudinal biomarker of the trial will be analyzed using a linear mixed-effects model to account for the correlation of repeated measurements within individuals. The model will include treatment group (dexmedetomidine vs. placebo), time (modeled as categorical variable with levels corresponding to baseline, 6h, 12h, 24h, 48h, 72h), and the treatment-by-time interaction as fixed effects. The primary effect of interest is the treatment by time interaction, which assesses whether the temporal evolution of NSE or other biomarkers differs between the treatment groups. A subject-specific random intercept for each subject will be included to account for between-subject variability. If supported by the data, a

	random slope for time may additionally be incorporated to allow for subject-specific variation in trajectories. Given the expected non-linear time course of the biomarkers of interest following resuscitation, time will be modeled as a categorical variable in the primary analysis, allowing for a flexible assessment of group differences at each clinically relevant time point without imposing assumptions about the functional form of the trajectory over time. As a sensitivity analysis, time will additionally be modeled as a continuous variable using flexible functional forms (e.g., restricted cubic splines) to account for unequal spacing between measurements and to capture potential non-linear trends over time. Model assumptions including approximate normality of residuals and homoscedascity of residual variance, will be assessed using graphical diagnostics. If required, appropriate transformations (e.g., logarithmic transformation of biomarkers concentrations) will be applied. The linear mixed-effect modeling approach allows to use all available observations under the assumption that data are missing at random without the need for imputation of missing outcome measurements. However, because death before 72 hours precludes measurement of NSE and may be related to disease severity, it will not be treated as missing data in the primary analysis but will instead be incorporated into the ranked composite endpoint. In the longitudinal analysis, all available NSE measurements obtained before death or last assessment will be included. This approach provides valid inference under a missing-at-random assumption for incomplete repeated measurements; however, because missingness due to death may be informative, results of the longitudinal analysis will be interpreted as supportive. Among patients who are alive at 72 hours, missing NSE values at that time point will be assumed to be missing at random, and analyses will be based on available outcome data without imputation. Sensitivity analyses will include evaluation of NSE concentrations at 72 hours among survivors and assessment of the robustness of the findings to alternative assumptions regarding missing data. Further analyses may include comparison of biomarker concentrations at certain time points by the non-parametric Mann Whitney U test, as well as comparison of AUCs of biomarkers. Clinical endpoints will be dichotomized into favorable and unfavorable neurological outcome (CPC 1-2 vs. CPC 3-5) and compared between the dexmedetomidine group and the placebo group using a Chi^2 test (or Fisher's exact test, as applicable) for the analysis at day 30 and at day 90. The same analysis will be performed for the comparison of the mRS (mRS 0-2 favorable outcome, mRS 3-6 unfavorable outcome), day 30 and 90 mortality, and occurrence of acute kidney injury or hypoxic hepatic injury within 72 hours of cardiac arrest. Mortality data will also be analyzed using time-to-event methods. Survival curves will be estimated using the Kaplan Meier method. Differences between treatment groups will be analyzed using the log-rank test. Furthermore, we will use Cox proportional hazard models, both univariate and multivariate (adjusted for traditional prognostication factors of CPR such as age, sex, time to

	sustained circulation, initial heart rhythm (shockable vs. non-shockable), MCS, etc.). Proportional hazards assumption will be checked. All analyses will be conducted including all patients, in whom the interventional treatment was initiated ("Modified Intention-to-Treat"). In a second step, we will perform these analyses in the two subgroups: patients with and without eCPR. Furthermore, we will perform these analyses in the "per-protocol" population defined as those patients, who received the interventional treatment for the foreseen period without dose adjustments. Dexmedetomidine pharmacokinetics and factors potentially influencing dosing will be assess using nonlinear mixed-effects modelling ('population pharmacokinetic modelling and simulations'). This approach allows to determine pharmacokinetic parameters characterizing the disposition and elimination of the drug (e.g., clearance, volume of distribution) for the typical ("mean") patient in the population and subpopulations, as well as simultaneously for each individual patient, thus allowing to quantify and explain interindividual variability aside from residual unexplained variability. Furthermore, the developed model enables simulations to assess the adequacy of standard doses administered in the study and alternative, optimized dosing regimens for the critically ill population and subpopulations. Quality-of-life outcomes (EQ-5D-5L index, EQ visual analogue scale, Hospital Anxiety and Depression Scale [HADS], Short Form-36, and Barthel Index) will be analyzed as exploratory endpoints. Continuous questionnaire scores will be analyzed using linear regression models with treatment group as the independent variable. If model assumptions are not met, non-parametric methods will be applied. Effect estimates with 95% confidence intervals will be reported. Because these analyses are exploratory, no adjustment for multiple comparisons will be performed. Safety data will be presented using descriptive statistics.
CONSENT	This study will enroll patients in an emergency setting. Because all study participants will be comatose at inclusion, deferred informed consent will be utilized. Consent will be sought from the study participant – or their legally authorized representative - as soon as their clinical condition allows.
DATA MONITORING COMMITTEE	There will be no data monitoring committee for this study.
DISCONTINUATION CRITERIA	The intervention may be stopped in the individual study participant, if the treating physicians and/or the investigators deem that continuation is against the best interest of the study participant. This may include a clinically relevant bradycardia and/or a deterioration of hemodynamics that is considered to be related to the intervention and that cannot be managed otherwise. The principal investigator, the deputy investigator, sub-investigators, as well as the sponsor representative will convene to discuss all cases individually and consider possible termination of the trial, if termination

	of the study drug is necessary because of adverse events deemed to be related to the study drug in >25% of all study participants (i.e., 8 of 30, 16 of 60, 23 of 90 patients). The decision must be unanimous, contain explanations why the decision to continue or discontinue the study was taken, and documented as a note-to-file. Importantly, discontinuations for other reasons (e.g., death of a patient due to a non-related cause) will not trigger such a meeting. Screening for discontinuation criteria (Adverse events of specific interest) will be conducted after each quarterly milestone of included patients (i.e., after 30, 60, and 90 enrolled patients). These safety analyses will be conducted in a blinded manner, unless unblinding is necessary.

3 LIST OF ABBREVATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
AESI	Adverse Event of Specific Interest
ALT	Alanin Aminotransferase
ATP	Adenosine Tri-Phosphate
AST	Aspartate Aminotransferase
CPC	Cerebral Performance Categories
CRF	Case Report Form
CRO	Clinical Research Organization
CSR	Clinical Study Report
CTIS	Clinical Trials Information System
CTR	Clinical Trial Regulation (REGULATION (EU) No 536/2014)
DOH	Declaration of Helsinki
DSUR	Development Safety Update Report
ECG	Electrocardiography
eCPR	Extracorporeal Cardiopulmonary Resuscitation
EOS	End of Study
EU	European Union
EVWeb	Eudravigilance system
GCP	Good Clinical Practice
GGT	Gamma-Glutamyltransferase
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCG	Human Corionic Gonadotropin
HVC	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ISO	International Standardisation Organization
KKS	Koordinationszentrum für Klinische Studien
LLT	Lower level terms
mm	Millimeter

mmol/l	Millimol/Liter
RBC	Red Blood Cells
SAE	Serious Adverse Event
SAP	Statistical analysis plan
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
VA-ECMO	Veno-Arterial ExtraCorporeal Membrane Oxygenation
WBC	White Blood Cells
WHO	World Health Organization

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Table 1 VISIT AND ASSESSMENT SCHEDULE

PERIODS	Name	SCREENING	TREATMENT							FOLLOW-UP 30/90 days	
	Duration										
VISITS	Number	1	2	3	4	5	6	7	8	9	10
	Name	Screening	Randomization	Start of Treatment	Control 1	Control 2	Control 3	Control 4	Control 5 = EOS	Follow-up 1 [3]	Follow-up 2
	Time	Day 0	Day 0	<2h of Admission	6h±2h after treatment	12h ± 3h after treatment	24h±4h after treatment	48h±6h after treatment	72h ±6h after treatment	Day 30 +7 Days	Day 90 + 14 Days
Informed Consent*		X									
Inclusion / Exclusion Criteria		X									
Medical History		X									
Concomitant/change in medication		X	X	X	X	X	X	X	X		
Physical Examination		X							X		
Body weight and height		X							X		
Vital Signs (BP, PR)		X	X	X	X	X	X	X	X		
12-lead ECG		X							X		
Laboratory Tests#				X	X	X	X	X	X		
Pregnancy Test		X									
HRQOL		X									X
Clinical Performance Scores			X				X	X	X	X	X
Adverse Events			X	X	X	X	X	X	X		
Treatment active				X	X	X	X				

*Informed consent is waived and will be sought as soon as possible.

#Blood samples for pharmacokinetic analyses are drawn at different time points

5 BACKGROUND INFORMATION

5.1 Background

The current guidelines on cardiopulmonary resuscitation (CPR) published in 2025 by the European Resuscitation Council state that post-resuscitation care should be started immediately after achieving sustained return of spontaneous circulation (ROSC) (1). Post-cardiac arrest syndrome encompasses hypoxic-ischaemic brain injury, post-cardiac arrest myocardial dysfunction, the systemic response to ischemia-reperfusion injury, and a possibly persisting triggering pathology (2-4). In short, the guidelines recommend to (i) treat the underlying cause, e. g. perform a coronary angiography when there is a high probability of an acute coronary occlusion, (ii) perform early computed tomography (CT) imaging to diagnose extra-cardiac causes of cardiac arrest (iii) stabilize respiration, hemodynamics and heart rhythm, (iv) perform temperature control, (v) treat seizures, if required, and (vi) perform general intensive care management (2). The ultimate goal of post-resuscitation care remains to improve patient outcomes and organ function. Importantly, poor neurologic function remains the main prognosis-limiting factor (5). According to the guidelines, several studies have attempted different approaches to improve neurological outcome after CPR, however, so far without success (2, 6-9). In conclusion, there is no specific treatment available that successfully ameliorates post-cardiac arrest syndrome as a whole, or hypoxic brain injury as the main prognosis-limiting determinant.

Dexmedetomidine is a highly selective α2-adrenoceptor agonist approved for the sedation of adult patients in intensive care units (ICU) (10). It induces a rather light sedation, so that patients are easy to rouse (10). Dexmedetomidine has shown analgesic effects in healthy volunteers and opioid-sparing effects in critically ill patients (10). It has sympatholytic activity and causes reduced catecholamine concentrations that translate into a lower heart rate and a lower blood pressure (10), which are the most common side effects of dexmedetomidine.

Apart from these well-known effects, numerous preclinical and some clinical trials provide evidence that dexmedetomidine may also exert neuroprotective effects and stabilize the blood brain barrier (BBB). For instance, Liu et al. demonstrated in a rat study of middle cerebral artery occlusion that dexmedetomidine markedly improved neurologic function and reduced infarct size, the inflammatory response as well as cerebral edema by stabilizing the BBB (11). Additionally, dexmedetomidine induced overexpression of CCN1 (cysteine-rich protein 61), a key regulator of cell adhesion, migration, proliferation and differentiation (including immune cells, endothelial cells, etc.) and a modulator of biological processes such as angiogenesis and wound healing (11). Interestingly, dexmedetomidine was shown to reduce inflammation and to improve the permeability of cell monolayers by upregulation of tight junction proteins in a transepithelial electrical resistance (TEER) model (12). In a murine model of myocardial ischemia and reperfusion, dexmedetomidine protected the mice from brain damage, diminished the disruption of the BBB, reduced neuronal edema formation, alleviated the local inflammatory response, reduced the activation of microglia, and improved cognitive function (13). The authors further reported an upregulation of the hypoxia-inducible factor 1a (HIF-1α) in the mice undergoing ischemia-reperfusion injury – a mechanism to protect from cerebral injury. Intriguingly, dexmedetomidine further upregulated this response, whereas its protective effects were attenuated in HIF-1α knock out mice (13). In a porcine cardiac arrest model, dexmedetomidine reduced biomarkers of cardiac and neurological injury, improved cardiac function (assessed by ejection fraction and stroke volume), improved neurologic function and reduced the inflammatory response (14). In this study, the authors compared two doses of dexmedetomidine: the 0.5µg/kg/h dose was superior to the 0.25µg/kg/h dose in reducing neurologic and organ injury. Importantly, both doses are in the lower ranges of the approved doses of 0.2-

1.4µg/kg/h (15), assuming comparable pharmacokinetics between humans and swine. In summary, dexmedetomidine stabilized the BBB in various preclinical models of ischemia reperfusion injury and was neuroprotective. Interestingly, it was also shown that dexmedetomidine may reduce myocardial injury in cardiac ischemia reperfusion injury models, indicating that these beneficial effects may not be limited to the BBB (16-18).

In humans, a randomized controlled trial showed that dexmedetomidine significantly reduced biomarkers of cardiac injury (troponin I) and of inflammation (tumor-necrosis factor alpha (TNF-α)) in patients undergoing cardiac valve replacement surgery under cardiopulmonary bypass (19). Gao et al. reported that pre-, post- and whole-course treatment with dexmedetomidine during cardiac valve replacement reduced biomarkers of cardiac injury and TNF-α significantly (20). These findings were confirmed in patients undergoing video-assisted thoracoscopic surgery (15, 21), in patients undergoing surgery for Stanford A dissection (22) or aortic surgery for various reasons (23), and in patients receiving a coronary artery bypass graft (24). In these randomized controlled trials, mostly a dose of 0.5µg/kg/h was administered.

5.2 Study rationale

We hypothesize that dexmedetomidine protects from ischemia-reperfusion injury and that it may exert beneficial effects in patients after out-of-hospital cardiac arrest (OHCA). More specifically, we hypothesize that dexmedetomidine stabilizes the BBB and protects from neurologic injury, which is still the main prognosis-limiting factor after OHCA and for which no specific treatment is currently available. Furthermore, in patients with myocardial ischemia as a triggering condition, we hypothesize that dexmedetomidine may exert cardioprotective effects and reduce the cardiac injury induced by ischemia and reperfusion.

The rationale for this study is based on numerous preclinical studies and clinical trials demonstrating protective effects of dexmedetomidine in ischemia-reperfusion injury. We plan to conduct a randomized, double-blind, placebo-controlled trial in patients who were successfully resuscitated after experiencing an OHCA and who achieved sustained circulation by either ROSC or extracorporeal cardiopulmonary resuscitation (eCPR). Including patients treated with eCPR, i.e., with resuscitative veno-arterial extracorporeal membrane oxygenation (VA ECMO), as a predefined subgroup is important, as its use is becoming increasingly common and patients requiring eCPR have a longer resuscitation duration, and therefore potentially a more pronounced ischemia-reperfusion injury (25).

Dexmedetomidine or placebo (0.9% sodium chloride solution) will be administered on top of standard of care. The typical drug combination to achieve adequate analgo-sedation in patients after OHCA consists of propofol, remifentanil and, possibly, rocuronium for the time of therapeutic hypothermic temperature management (typically 24 hours). Dexmedetomidine will be administered at a rate of 0.5 µg/kg/h, which on the one hand is the dose mostly used in the above-mentioned studies, and on the other hand is relatively low with regard to approved doses and therefore considered to be safe in critically ill patients with regard to hemodynamic side effects. The administration of dexmedetomidine will start as soon as possible after admission to the hospital (after an intravenous access has been established) and last for 24h. Thereafter, it will be at the discretion of the treating physicians to use dexmedetomidine. The pharmacokinetics (PK) of dexmedetomidine have been described in detail for a general critically ill patient population (26). However, there are no clinical data on its pharmacokinetics during target temperature management at 33° Celsius. A study in newborn piglets with cerebral hypoxia-ischemia undergoing target temperature management at 33.5° Celsius suggests a significantly reduced clearance of dexmedetomidine (27). Therefore, we will investigate whether the pharmacokinetics of

dexmedetomidine differ in this specific population, and compare the PK between the patients requiring eCPR and those who are treated with traditional CPR.

In this study, we will focus on the effects of dexmedetomidine on biomarkers of neurologic injury, including neuron specific enolase (NSE) and S100ß, as well as on markers of cardiac injury (troponin T) in study participants with acute coronary syndromes (i.e. those who require coronary intervention). However, apart from these well-established biomarkers, we will also investigate the effects of dexmedetomidine on clinical endpoints of neurological function after OHCA, as recommended by the European Resuscitation Council: the Cerebral Performance Categories (CPC) and the modified Rankin Score (mRS) on day 30 and 90. Furthermore, we will assess the frequency of acute renal or hypoxic hepatic injury between both groups.

The currently available data do not allow for a robust sample size calculation with realistic effect estimates for an endpoint-driven trial. In similar populations, approximately 40% of study participants had a good neurological outcome (9). At our institution, 46% of patients with sustained ROSC admitted during 2023-2024 had good neurologic outcome (n=267; unpublished data). In these two years, 96 patients required treatment with eCPR and 30% had a good neurologic outcome (unpublished data). An improvement from 40% to 50% would require a sample size of n=387 per group, an improvement from 40% to 55% would require a sample size of n=173 per group, and an improvement from 40% to 60% would require a sample size of n=97 per group. These differences in effect sizes have massive implications on the feasibility and the costs of such a trial (single center vs. multi-center). Thus, the current trial also serves as a preparation for an endpoint-driven trial that is based on robust estimates of the expectable effect size and therefore (i) has a higher likelihood of success and (ii) is cost-efficient.

To investigate the potential underlying mechanism, we will quantify the pro-inflammatory cytokine IL-6 and C-reactive protein concentrations. To investigate whether dexmedetomidine affects the permeability of blood vessels, we will investigate endothelial barrier function in vitro in transwells using fluorescent tracers with different molecular weight (FITC-Dextran) to trace potential leakage. We have shown earlier that vascular permeability is a hallmark of post-cardiac arrest syndrome (28). We will investigate whether patient plasma may impact on endothelial barrier function, whether these effects differ between the dexmedetomidine and the placebo group, but also whether dexmedetomidine may impact on the effects of several mediators known to disrupt the endothelial barrier (e.g. histamine, TNF-α, thrombin).

6 STUDY OBJECTIVES (HYPOTHESIS)

6.1 Primary objective (Hypothesis)

To investigate the effects of dexmedetomidine in patients after out-of-hospital cardiac arrest in comparison to placebo, as measured by the ranked composite of death before 72h and of neuron-specific enolase (NSE) 72h after cardiac arrest

6.2 Secondary objectives (Hypothesis)

- To investigate the effects of dexmedetomidine in patients after out-of hospital cardiac arrest in comparison to placebo, as measured by concentrations of neuron-specific enolase 72h after cardiac arrest and during the first 72h after cardiac arrest

- To investigate the effects of dexmedetomidine in patients after out-of-hospital cardiac arrest in comparison to placebo, as measured by the ranked composite of death before 72h and of S100ß 72h after cardiac arrest
- To investigate the effects of dexmedetomidine in patients after out-of-hospital cardiac arrest in comparison to placebo, as measured by concentrations of S100ß 72h after cardiac arrest and during the first 72h after cardiac arrest
- To assess the effects of dexmedetomidine on the occurrence of acute kidney injury or acute hepatic injury occurring up to 72h after cardiac arrest
- To assess the effect of dexmedetomidine on the extent of cardiac injury in patients with acute coronary syndromes as causative disease (i.e., those who require coronary interventions) up to 72h after cardiac arrest
- To assess the effects of dexmedetomidine on exploratory biomarkers up to 72h after cardiac arrest
- To investigate the underlying mechanisms of dexmedetomidine on endothelial barrier function by performing in vitro experiments using endothelial cell cultures
- To investigate the pharmacokinetics of dexmedetomidine in patients undergoing target temperature management at 33° Celsius

7 STUDY DESIGN

This is a randomized, double-blind, placebo-controlled clinical trial in patients with sustained circulation after OHCA. A total of 120 patients will be randomized to receive 0.5µg/kg/h dexmedetomidine or placebo (0.9% saline solution) for 24h.

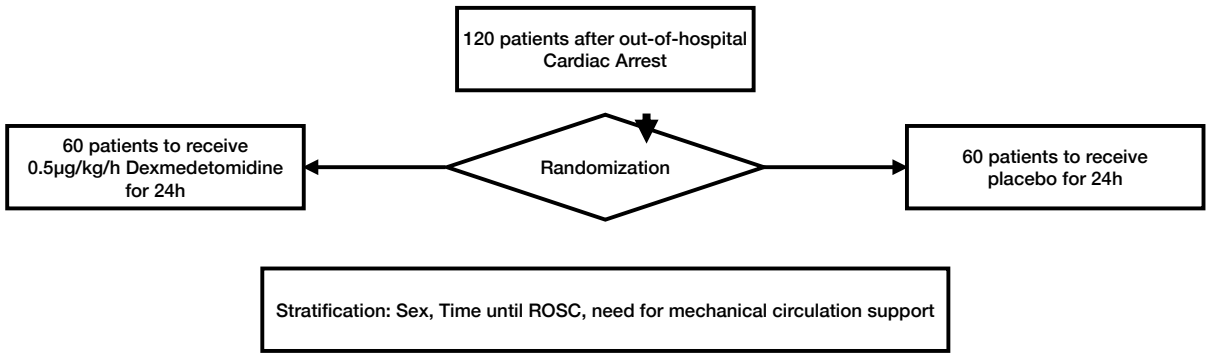

Figure 1. Flow Chart of the trial

7.1 Study population

Study participants will be recruited at the Department of Emergency Medicine immediately after admission. This is a single center study. In- and exclusion criteria will be checked as soon as information on each study participant becomes available. Inclusion of each study participant will also be discussed with treating physicians.

7.1.1 Inclusion criteria

- Adult patients >18 years of age experiencing out-of-hospital cardiac arrest

- Sustained circulation (defined as ROSC or successful eCPR flow lasting for at least 20 minutes) within 90 minutes of suspected collapse
- Comatose (Glasgow Coma Scale <8) on admission or suspected in patients receiving sedatives
- Start of the trial drug is possible within 2 hours of hospital admission
- Subjected to temperature control
- Combined no-flow and low-flow time of ≥10 minutes

7.1.2 Exclusion criteria

- Expected death within 72 hours or known medical condition limiting expected survival to < 3 months
- Known limitations to intensive care therapy and "Do Not Resuscitate" or "Allow Natural Death" orders
- Advanced directive of patient to decline resuscitation, intensive care measures, or participation in a clinical trial according to the local legal requirements of participating trial sites
- Known pre-arrest CPC of 3 or 4
- Known diseases that may interfere with biomarkers indicative of neurologic injury (e.g., cancer, inflammation, acute bleeding, stroke or infection of the central nervous system) at the discretion of the investigator
- Time to sustained circulation > 90 minutes
- No-flow time >10 minutes
- Unwitnessed cardiac arrest unless the no-flow time can safely be assumed to be <10minutes
- Unmanageable hemodynamic and respiratory instability albeit the use of vasopressors and/or mechanical circulatory support – at the discretion of the treating physicians
- Known allergies or intolerances against any of the substances used in the trial
- Requirement of acute surgery at screening
- Pregnancy or breastfeeding

7.1.3 Females of childbearing potential

A pregnancy test will be performed in all females of childbearing potential to rule out pregnancy. Pregnant women will not be included in the trial. Breastfeeding women will also not be included in the trial.

7.1.4 Subjects in an emergency situation

All study participants enrolled in this clinical trial will be in an emergency situation and comatose at the time of study inclusion. Consequently, deferred informed consent will be utilized at enrollment. However, informed consent will be obtained from each participant as soon as their clinical condition permits.

7.1.5 Study duration

For the individual patient, the treatment period lasts for 24h. The observation period lasts for 72h. The follow-up visits are planned at day 30 and day 90.

7.1.6 Withdrawal and replacement of subjects

Criteria for withdrawal

Subjects may prematurely discontinue from the study at any time. Premature discontinuation from the study means that the subject did not undergo an end of study examination as planned per protocol.

Subjects must be withdrawn under the following circumstances:

- at their own request

- if the Investigator feels it would not be in the best interest of the subject to continue. This may include a clinically relevant bradycardia and/or a deterioration of hemodynamics that is considered to be related to the intervention and that cannot be managed otherwise.

In all cases, the reason why subjects are withdrawn must be recorded in detail in the CRF and in the subject's medical records. Should the study be discontinued prematurely, all study materials (completed, partially completed and empty CRFs) will be retained.

Follow-up of patients withdrawn from the study

In case of premature discontinuation after study drug intake, the investigations scheduled for the EOS visit will be performed 48h after study drug discontinuation (72h ± 8h after cardiac arrest). The subjects will be advised that participation in these investigations is voluntary. Furthermore, they may request that from the time point of withdrawal no more data will be recorded and that all biological samples collected in the course of the study will be destroyed.

Replacement policy

Subjects may be replaced, if treatment is not initiated (e.g. exclusion criterion is met after inclusion, but before start of the infusion of the study drug).

7.1.7 Premature termination of the study

The sponsor has the right to close this study at any time. The IEC and the competent regulatory authority must be informed via CTIS within 15 days of early termination.

The trial or single dose steps will be terminated prematurely in the following cases:

- If adverse events occur which are so serious that the risk-benefit ratio is not acceptable.
- If the number of dropouts is so high that proper completion of the trial cannot realistically be expected.
- The principal investigator, the deputy investigator, sub-investigators, as well as the sponsor representative will convene to discuss all cases individually and consider possible termination of the trial, if termination of the study drug is necessary because of adverse events deemed to be related to the study drug in >25% of all study participants (i.e. 8 of 30, 16 of 60, 23 of 90 patients). The decision to continue must be unanimous, contain explanations why the decision to continue the study was taken, and documented as a note-to-file. Importantly, discontinuations of the interventional drug for other reasons (e.g. death of a patient due to a non-related cause) will not trigger such a meeting. Cases will not be unblinded for that discussion unless it is absolutely necessary.
- Screening for discontinuation criteria will be conducted after each quarterly milestone of included patients (i.e., after 30, 60, and 90 enrolled patients).

8 METHODOLOGY

8.1 Study medication

Intervention:

Active agent and characteristics: dexmedetomidine

Trade name of the agent: **Dexdor**

Manufacturer: Orion Pharma

Drug supply: Central Pharmacy of the Vienna General Hospital

Storage Instructions: room temperature

Authorization status: authorized

Control:

Active agent and characteristics: 0.9% Sodium Chloride solution

Trade name of the agent: Kochsalz "Braun" 0.9% Infusionslösung

Manufacturer: B. Braun Melsungen AG

Drug supply: Central Pharmacy of the Vienna General Hospital

Storage Instructions: room temperature

Authorization status: authorized

8.1.1 Dosage and administration

Initial dose: 0.5µg/kg/h

Maintenance dose: 0.5µg/kg/h

Route and mode of administration: intravenous infusion

Duration: 24h

8.1.2 Study-drug up- and down titration

Dexmedetomidine may cause bradycardia and hypotension, which may be aggravated by the underlying disease and/or target temperature management. Hypotension may be treated with an increased dose of noradrenaline, which is typically required in almost all patients after OHCA treated with temperature control. In some patients, dobutamine infusion is also required and the dose may be adapted or treatment may be started to stabilize patients. However, if catecholamine doses are already high or if the condition of the patient precludes treatment with dobutamine (e.g. in case of clinically relevant electrical instability) or further increases of the doses, dexmedetomidine may either be reduced to 0.25µg/kg/h or stopped (temporarily or permanently). This will be decided by the treating physicians.

Up-titration is not planned within the first 24h and not deemed necessary.

8.1.3 Study drug interruption or discontinuation

The Investigator must temporarily interrupt or permanently discontinue the study drug if continued administration of the study drug is believed to be contrary to the best interests of the patient.

The interruption or premature discontinuation of study drug might be triggered by an AE, a diagnostic or therapeutic procedure, an abnormal assessment (e.g., laboratory abnormalities), or for administrative reasons, in particular withdrawal of the patient's consent.

The reason for study drug interruption or premature permanent discontinuation must be documented in the CRF.

8.1.4 Study drug interruption

The study drug may be temporarily interrupted in case of an AE (e.g. hemodynamic instability). However, it may be restarted if treating physicians decide that restarting the study drug may be safe. For instance, if the reason for hemodynamic instability (other than the study drug) has been successfully treated. Any interruptions must be documented.

8.1.5 Study drug premature permanent discontinuation

Study drug premature permanent discontinuation due to an adverse event

If the reason for premature permanent discontinuation of study treatment is an AE, the patient should have a "Premature End of Study (EOS)" visit with all the assessments performed before the study drug discontinuation, whenever possible.

Study drug premature permanent discontinuation due to another reason than adverse event

If the reason for premature permanent discontinuation of study treatment is not an AE, the patient should be withdrawn from the study (withdrawal of consent) and have the end of study (EOS) visit with all the assessments performed before the study drug discontinuation, whenever possible.

8.1.6 Study-drug delivery & drug storage conditions

The study drug and the placebo may be kept at room temperature. Once diluted, dexmedetomidine should be used as soon as possible, but at maximum within 24h. The central pharmacy of the General Hospital of Vienna will supply the study team with the study drug. However, it will be prepared at the study ward by staff not otherwise involved in the trial conduct.

8.1.7 Study drug packaging and labeling

The labels of the study drug must contain: name, address and telephone number of the sponsor, Clinical Trial Reference/Protocol number; trial subject identification code, randomization number, "dexmedetomidine or placebo", number of the batch, expiry date, dose to be administered, route to be administered, the text "for clinical trial use only", storage conditions.

8.1.8 IMP administration & handling

The study drug must be started within 2h of hospital admission and within 24 hours of dilution.

8.1.9 Drug accountability

Drug Accountability will be recorded at on-going basis on paper form/source data, Drug dispensing have to be entered into the CRF. Furthermore, the correct administration of the IMP or any variations concerning that will be recorded in the CRF.

8.1.10 Procedures to assess subjects' compliance

Not applicable, single infusion for 24h in comatose patients.

8.2 Concomitant medication

The well-being of the patient has the first priority, and modifications of concomitant treatment during the trial are allowed as necessary. They will be documented in the patient's records.

Allowed: any concomitant treatment necessary**.**

Not allowed: Dexmedetomidine use within 24h. Of note, this drug is usually not used during the first 24h after ROSC, because patients are often analgo-sedated using other drugs and/or receive muscle relaxants.

8.3 Randomization and stratification

After checking for eligibility, patients will be randomized using a web-based randomization software available at www.meduniwien.ac.at/randomizer. Patients will be stratified based on (i) time to ROSC (within 30 minutes and > 30 minutes) and (ii) necessity of mechanical circulatory support. We will use

permuted blocks randomization for this study. Randomization will be conducted by study staff having access to these data. However, only blinded study staff will receive the result of the randomization process.

8.4 Blinding

The study drugs will be prepared by staff neither involved in the conduct of the study nor in the treatment of patients. Dexmedetomidine is not distinguishable from placebo based on its physicochemical appearance (colorless fluid).

Study participants, clinicians and nurses, as well as study staff will be blinded to group allocation of patients. Treatment stops after 24h.

8.4.1 Emergency procedure for unblinding

After web-based randomization, group allocation will be printed and put into a sealed envelope that is labeled with the study name, patient ID, contact details of the investigators and empty spaces for the time and date of unblinding, as well as reason for unblinding. At the end of the treatment period the envelope will be collected from the study team and kept until the entire trial has finished. Unblinding must only be done, if the safety and well-being of the patient necessitates it.

8.4.2 Unblinding at the end of the study

At the end of the study, after the last visit of the last patient unblinding will take place. This will be done by the unblinded study team (e.g. study pharmacist), who has access to the web-based randomization software.

8.5 Benefit and risk assessment

The potential benefits of this study for individual study participants, but also for the entire group of patients, are improved neurological function. A specific treatment that may improve neurological outcome after cardiac arrest is desperately required. As mentioned, poor neurologic function is still the main prognosis-limiting factor in these patients (2). Data also hint towards cardioprotective effects of dexmedetomidine (14). Of note, the most frequent triggers of cardiac arrest are of cardiac nature – both due to ischemia and due to arrhythmia. Thus, the subgroup of patients in whom a myocardial infarct triggered cardiac arrest may specifically benefit from participation in this study. Besides, evaluation of standard and potentially more beneficial dosing regimens of dexmedetomidine will contribute to more effective and safe dosing in critically ill patients.

However, dexmedetomidine also has side effects that may put risks on patients. First and foremost, dexmedetomidine impacts on the hemodynamics of patients by reducing endogenous catecholamine concentrations and consequently reducing the heart rate and the blood pressure of patients. Patients, who have undergone CPR, may be hemodynamically stable, but the majority of patients requires fluid substitution and catecholamine treatment to achieve stable hemodynamics and maintain target blood pressure (2). Apart from the triggering condition that may impact cardiovascular stability, standard analgo-sedation includes propofol, which is also well-known to induce hypotension (29). Furthermore, heart rate and blood pressure may also drop during hypothermic target temperature management after CPR. Finally, after CPR vascular permeability typically increases due to ischemia and reperfusion injury and patients typically require fluid substitution (28). The hemodynamic effects of dexmedetomidine are dose-dependent and hence, administering a bolus infusion of dexmedetomidine is not recommended in critically ill patients (15). Furthermore, hemodynamic effects of dexmedetomidine are reported to be

manageable, either by reducing its dose or by using anticholinergic medication (for bradycardia) as well as by intensifying catecholamine support (for hypotension). The summary of product characteristics (SMPC) suggests to use anticholinergic agents such as atropine to treat bradycardia (15). The ERC guidelines for resuscitation suggest not to treat bradycardia unless signs of hypoperfusion occur (such as increasing lactate concentrations or reduced urinary output), even if heart rates are only 30-40/min. Preclinical data suggests improved diastolic function for low heart rates. Clinical data from patients after resuscitation who were normothermic support these observations, while there are no data on that topic in patients undergoing therapeutic temperature management at lower temperatures (1).

Within this trial, we aim to include patients who are "hemodynamically stable", that is, patients who do not require escalating doses of catecholamines or vasopressors, and eCPR patients who achieve ECMO flow without excessive fluid administration, as determined by the treating physician. Furthermore, although from a pharmacological point-of-view a bolus infusion would be desirable in order to achieve the effects of dexmedetomidine as soon as possible, this would possibly put patients at risk of deteriorating hemodynamically and therefore a bolus infusion will not be administered. The chosen dose of dexmedetomidine is relatively low, given that approved doses range from 0.2-1.4 µg/kg/h. Given that the hemodynamic effects of dexmedetomidine are dose-dependent, we believe that they should be manageable with the above-mentioned means. All patients will be treated at an intensive care unit and will be monitored continuously. Any hemodynamic alterations will therefore be noticed immediately. This is also true for other side effects, e.g. cardiac arrythmia. Other side effects will play a smaller role within the setting of an ICU, such as hyper- or hypoglycemia, respiratory depression, or agitation. Importantly, it is at the discretion of the treating physicians to reduce the dose and/or terminate treatment with dexmedetomidine (or placebo), if it is contrary to the best interest of the patients. That said, a reduction in the administered propofol dose, or other agents that may cause hypotension, may also be attempted.

In our opinion, the potential benefits of the study – improving the most prognosis-limiting condition after cardiac arrest – outweigh the risks, which, while serious, are well-known to physicians and are generally considered manageable.

8.6 Study procedures

8.6.1 General rules for trial procedures

- All study measures like blood sampling and measurements (vital parameters, ECG, etc.) have to be documented with date (dd:mm:yyyy).
- In case several study procedures are scheduled at the same time point, there is no specific sequence that should be followed.
- The dates of all procedures should be according to the protocol. The time margins mentioned in the study flow chart are admissible. If for any reason, a study procedure is not performed within scheduled margins a protocol deviation should be noted, and the procedure should be performed as soon as possible or as adequate.
- If it is necessary for organizational reasons, it is admissible to perform procedures which are scheduled for one visit at two different time points. Allowed time margins should thereby not be exceeded.

8.6.2 Recruitment procedure

Patients will be recruited at the Department of Emergency Medicine. Inclusion of patients will be discussed with the treating physicians. All included patients will be comatose and informed consent will be waived. Patients will be informed about study participation as soon as possible.

8.6.3 Screening investigation

Screening will mostly be based on the available clinical information. In- and exclusion criteria will be checked based on available information from the Emergency Medical Services and from potential bystanders. Furthermore, electronic health records will be checked to gain as much information as possible on the patient. However, screening must be quick in order to enable a rapid initiation of treatment. Weight and height will be measured or estimated. If in- and exclusion criteria are met and if treating physicians agree to include the patient, study staff will proceed with randomization.

8.6.4 Treatment Phase

If eligible for study participation, patients will be randomized by study staff. Study staff not otherwise involved in the conduct of the trial will prepare the study drug or placebo, as applicable. Infusion of the study drug will start as soon as possible, but within 2h of admission to the hospital. Treatment will last for 24h and will be stopped thereafter. If treatment with dexmedetomidine is indicated at any point after 24h, it will be at the discretion of the treating physicians.

A baseline blood sample will be drawn. An ECG will be written. Arterial blood gas analysis will be done repetitively as part of clinical routine, but will also be done at the planned blood sampling time points for biomarker quantification: baseline, 6h (±2 h), 12h (±3h), 24h (±4h), 48h (±4h), and 72h (±4h) after initiation of the study drug.

For pharmacokinetic analyses, we will draw a maximum of nine blood samples in each patient. The terminal elimination half-life of dexmedetomidine is approximately 1.5h (9), but may be longer in patients undergoing target temperature management at 33°Celsius (33). Importantly, drawing of blood samples may sometimes not be possible due to the clinical condition of a patient (e.g. interventions, surgeries, etc). However, we plan to draw one blood sample at baseline (before start of infusion, one in the very early phase of treatment (i.e. 5-20 min after start of treatment), one before steady state (2-6h), two during steady state (one 12- 18h after start of treatment and one right before the end of infusion) and four blood samples after cessation of treatment (2 samples 5-30 min, 1 sample 1-4h, 1 sample 6-12h, and one sample 24h after infusion after end of treatment). For modeling purposes, it is essential to meticulously document the start of infusion, any interruptions and dose adjustments of the dexmedetomidine infusion, termination of treatment and the timing of blood sampling. Whole blood will be centrifuged at 2500g, 4°Celsius for 15 minutes.

8.6.5 Observation Period

During the active period we will perform visits to the patient after 6h (±2h), 12h (±3h), 24h (±4h), 48h (±4h) and 72h (±4h) to perform blood sampling for biomarkers. The blood sampling procedures for pharmacokinetic analyses after cessation of treatment are explained above and will also fall in this period.

This period will also delineate the safety and tolerability period during which any changes in medication will be documented and during which any adverse events will be documented. The terminal elimination half-life of dexmedetomidine is about 1.5h. After 4 half-lives a drug is considered to be cleared from circulation. However, as there are no data on the pharmacokinetics of dexmedetomidine in this specific population, longer half-lives are possible. An active surveillance period of 48h after cessation of treatment is considered sufficient to capture any safety related problems caused by dexmedetomidine.

8.6.6 End-of-study (EOS) examination

The EOS examination will be performed after 72h. It includes a physical examination, a concise documentation of all adverse events. Serious adverse events, severe adverse events and those deemed

related with the study drug will be followed up closely. This is a very sick patient population and numerous adverse events are expectable.

8.6.7 Follow-up Visits

On day 30 and day 90 we will contact patients to document their neurologic state using the CPC and mRS scales (e.g. by telephone, by visits in hospital, etc). Furthermore, day 30 and 90 mortality will be documented (e.g. by discharge documents, electronic health records, mortality register). On day 90 we will perform quality of life questionnaires (over the phone, see appendices).

8.6.8 Laboratory Assays

Biomarkers indicative of neurologic injury:

Neuron Specific Enolase (NSE) and S100ß will be quantified in the central laboratory of the Vienna General Hospital. Hemolysis may increase neuron specific enolase parameters, because erythrocytes also contain this enzyme. Thus, quantification of hemolysis is important for the interpretation of NSE concentrations. Of note, markers of hemolysis (e.g., LDH, haptoglobin, hemolysis index, bilirubin) are quantified at least twice daily in clinical routine, which will allow to assess actual hemolysis in patients.

Biomarkers indicative of cardiac injury:

Troponin T will be quantified in the central laboratory of the Vienna General Hospital.

Biomarkers indicative inflammatory reaction:

C-reactive protein and Interleukin (IL)-6 will be quantified in the central laboratory of the Vienna General Hospital as part of routine of care.

Acute kidney injury:

Creatinine concentrations are quantified as part of routine clinical care. Acute kidney injury is defined by KDIGO criteria (Kidney disease: Improving Global Outcomes) (30). A mild acute kidney injury is defined by a 50-90% increase in creatinine concentrations from baseline or an increase that is ≥0.3mg/dL in absolute values or a reduction of urine volume <0.5mL/kg/h for 6 to 12 hours. A moderate acute kidney injury is defined by a 100-190% increase in creatinine concentrations from baseline or a reduction in urine production to <0.5mL/kg/h for >12 hours. A severe kidney injury is defined by an ≥300% increase in creatinine concentrations from baseline or an increase of serum creatinine ≥4.0mg/dL or the requirement of renal replacement therapy. Furthermore a reduction of urine production to <0.3ml/kg/h or anuria for ≥12 hours defined severe acute kidney injury. Urine volume is regularly documented by the clinical team in the electronic health records.

Hypoxic liver injury:

We use Henrion's (31) criteria for hypoxic liver injury, which defines hypoxic liver injury as an >20-fold increase of the upper limit of normal for aminotransferase levels. These levels will be quantified at least once daily as part of routine blood sampling on intensive care units.

In vitro assays of endothelial function:

To investigate the effects of dexmedetomidine on the permeability of blood vessels, we will investigate endothelial barrier function in transwells using fluorescent tracers with different molecular weight (FITC Dextran) to trace potential leakage. Cultured endothelial cells will be cultured with patient serum

(baseline, 4h, 24h, 72h) from the placebo and the dexmedetomidine group to investigate (i) whether patient plasma may increase endothelial permeability in vitro and (ii) to investigate potential group differences. Co-stimuli (e.g., hypoxia) and inhibitors will be applied to investigate the underlining mechanisms combined with mRNA and protein analysis. These experiments will be done at the University of Graz.

Pharmacokinetics:

Drug concentrations will be quantified using tandem liquid mass spectrometry at the central laboratory of the Vienna General Hospital. Plasma samples will be stored for batch analysis.

Required blood samples:

For pharmacokinetic analysis a 3mL EDTA-anticoagulated blood sample will be drawn. The maximum number of samples will be 9 samples per patient:

- 1 sample at baseline – before start of treatment
- 1 sample between 5-20 minutes of treatment (early phase)
- 1 sample between 2- 6 hours of treatment (before steady state)
- 1 sample between 6-12 hours of treatment (steady state)
- 2 sample 5-30 minutes after cessation of treatment
- 1 sample 1-4h after cessation of treatment
- 1 sample 6-12h after cessation of treatment
- 1 sample 24h after cessation of treatment

In total, a maximum of 27mL blood will be required for pharmacokinetic analysis

For exploratory biomarker analysis blood sampling is required at baseline, after 6h, 12h, 24h, 48h, and 72h. For that matter, one EDTA- (6mL), one citrate-anticoagulated blood sample (3.5mL) and one serum tube (3mL) will be drawn. In sum, 102mL of blood will be required for all analyses. Of note, part of the analyses will be done within routine laboratory investigations.

Blood samples for pharmacokinetic analyses, but also for exploratory biomarkers will be centrifuged at 2,500 g for 15 minutes at 4 degrees. Supernatant will be transferred into 500µL containing aliquots and stored for batch analysis.

In case plasma samples are not required for the foreseen quantifications, they may be used for the quantification of other exploratory biomarkers.

In total, 84mL blood will be drawn over a period of 72h.

8.6.9 Definition of the end of the trial

The end of the trial is defined as the date of the last visit of the last patient undergoing the trial.

9 SAFETY DEFINITIONS AND REPORTING REQUIREMENTS

9.1 Adverse events (AEs)

9.1.1 Summary of known and potential risks of the study drug

The Summary of Product Characteristics of dexmedetomidine lists the following adverse events with the following frequencies (very common ≥1/10, common (≥1/100 - <1/10), occasionally (≥1/1.000 - <1/100), rare (≥1/10.000 - < 1/1.000), very rare (<1/10.000), unknown (15).

Endocrine disorders:

- unknown: diabetes insipidus

Metabolism and nutritional disorders:

- common: hyperglycemia, hypoglycemia;
- occasionally: metabolic acidosis, hypoalbuminemia

Psychiatric disorders:

- common: Agitation
- occasionally: hallucinations

Cardiac disorders:

- very common: bradycardia
- common: myocardial ischemia or infarction, tachycardia
- occasionally: atrioventricular block, reduced cardiac output, cardiac arrest

Vascular disorders:

- very common: hypotension, hypertension

Respiratory disorders, disorders of the chest and the mediastinum:

- very common: respiratory depression
- occasionally: dyspnea, apnea

Gastrointestinal disorders

- very common: nausea, emesis, xerostomia
- occasionally: meteorism

General disorders and administration site disorders:

- common: hyperthermia, withdrawal symptoms
- occasionally: thirst, ineffectiveness of the drug

Dexmedetomidine may reduce the blood pressure and cause bradycardia by central sympathetic blockade. As target temperature management also causes bradycardia, synergistic effects must be considered. The ERC guidelines discuss bradycardia as a side-effect of target temperature management and recommend to only treat bradycardia if there is a risk of hypoperfusion (e.g. increase of lactate concentrations, reduced urinary output) even if the heart rate drops to 30-40/minute (1). The SMPC report that bradycardia and/or hypotension may be treated with anticholinergic substances (such as

atropine or glycopyrrolate). In case of bradycardic arrhythmias, it is recommended to stop treatment (15).

9.1.2 Adverse Events of Specific Interest

AESI include clinically significant bradycardia or hemodynamic instability that require dose reductions or temporary of permanent interruptions of the interventional drug. AESI do not include hemodynamic instabilities explained by other circumstances and do not include dose adjustments of catecholamines or fluid substitution within regular care.

9.1.3 Definition of adverse events

An AE is any untoward adverse change from the subject's baseline condition, i.e., any unfavorable and unintended sign including an abnormal laboratory finding, symptom or disease which is considered to be clinically relevant by the physician that occurs during the course of the study, whether or not considered related to the study drug.

Adverse events include:

- Exacerbation of a pre-existing disease.
- Increase in frequency or intensity of a pre-existing episodic disease or medical condition.
- Disease or medical condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study.
- Continuous persistent disease or symptoms present at baseline that worsen following the start of the study.
- Lack of efficacy in the acute treatment of a life-threatening disease.
- Events considered by the Investigator to be related to study-mandated procedures.
- Abnormal assessments, e.g., ECG and physical examination findings, must be reported as AEs if they represent a clinically significant finding that was not present at baseline or worsened during the course of the study.
- Laboratory test abnormalities must be reported as AEs if they represent a clinically significant finding, symptomatic or not, which was not present at baseline or worsened during the course of the study or led to dose reduction, interruption or permanent discontinuation of study drug.

Adverse events do not include:

- Pre-planned interventions or occurrence of endpoints specified in the study protocol are not considered AE´s, if not defined otherwise (eg.as a result of overdose)
- Medical or surgical procedure, e.g., surgery, endoscopy, tooth extraction, transfusion. However, the event leading to the procedure is an AE. If this event is serious, the procedure must be described in the SAE narrative.
- Pre-existing disease or medical condition that does not worsen.
- Situations in which an adverse change did not occur, e.g., hospitalizations for cosmetic elective surgery or for social and/or convenience reasons.
- Overdose of either study drug or concomitant medication without any signs or symptoms. However, overdose must be mentioned in the Study Drug Log.

9.2 Serious adverse events (SAEs)

According to the Regulation (EU) No 536/2014 “Serious adverse event” means any untoward medical occurrence that at any dose

- requires inpatient hospitalization or prolongation of existing hospitalization,

- results in persistent or significant disability or incapacity,
- results in a congenital anomaly or birth defect,
- is life-threatening, or
- results in death.

9.2.1 Hospitalization – Prolongation of existing hospitalization

Hospitalization is defined as an overnight stay in a hospital unit and/or emergency room.

An additional overnight stay defines a prolongation of existing hospitalization.

The following is not considered an SAE and should be reported as an AE only:

- Treatment of an emergency on an out subject basis for an event not fulfilling the definition of seriousness given above and not resulting in hospitalization.

The following reasons for hospitalizations are not considered AEs, and therefore not SAEs:

- Hospitalizations for cosmetic elective surgery, social and/or convenience reasons.
- Elective treatment of a pre-existing disease or medical condition that did not worsen, e.g., hospitalization for chemotherapy for cancer, elective hip replacement for arthritis.

9.2.2 SAEs related to investigational drug

Such SAEs are defined as SAEs that appear to have a reasonable possibility of causal relationship.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

SUSARs are all serious adverse reactions with **suspected** causal relationship to the study drug that is **unexpected** (not previously described in the Summary of Product Characteristics or Investigator's brochure) and serious.

9.3 Pregnancy

Any pregnancy that occurs during study participation must be reported to the Investigator/sponsor. To ensure subject safety, each pregnancy must be reported to the Sponsor immediately. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or SAE. Spontaneous abortions must be reported as an SAE.

Any SAE occurring in association with a pregnancy brought to the Investigator's attention after the subject has completed the study and considered by the Investigator as possibly related to the investigational product, must be promptly reported to the Investigator/sponsor.

In addition, the Investigator must attempt to collect pregnancy information on any female partners of male study subjects who become pregnant while the subject is enrolled in the study. Pregnancy information must be reported to the Investigator/sponsor as described above.

9.4 Severity of adverse events

The severity of clinical AEs is graded on a three-point scale: mild, moderate, severe, and reported on specific AE pages of the CRF.

If the severity of an AE worsens during study drug administration, only the worst intensity should be reported on the AE page. If the AE lessens in intensity, no change in the severity is required.

If an AE occurs during a washout or placebo run-in phase and afterwards worsens during the treatment phase, a new AE page must be filled in with the intensity observed during study drug administration.

Mild

Event may be noticeable to subject; does not influence daily activities; the AE resolves spontaneously or may require minimal therapeutic intervention;

Moderate

Event may make subject uncomfortable; performance of daily activities may be influenced; intervention may be needed; the AE produces no sequelae.

Severe

Event may cause noticeable discomfort; usually interferes with daily activities; subject may not be able to continue in the study; the AE produces sequelae, which require prolonged therapeutic intervention.

A mild, moderate or severe AE may or may not be serious. These terms are used to describe the intensity of a specific event (as in mild, moderate, or severe myocardial infarction). However, a severe event may be of relatively minor medical significance (such as severe headache) and is not necessarily serious. For example, nausea lasting several hours may be rated as severe, but may not be clinically serious. Fever of 39°C that is not considered severe may become serious if it prolongs hospital discharge by a day. Seriousness rather than severity serves as a guide for defining regulatory reporting obligations.

9.5 Relationship to study drug

For all AEs, the Investigator will assess the causal relationship between the study drug and the AE using his/her clinical expertise and judgment according to the following algorithm that best fits the circumstances of the AE:

Not related

- May or may not follow a temporal sequence from administration of the study product
- Is biologically implausible and does not follow known response pattern to the suspect study drug (if response pattern is previously known).
- Can be explained by the known characteristics of the subject's clinical state or other modes of therapy administered to the subject.

Unlikely

- There is a reasonable temporal relation between the AE and the intake of the study medication, but there is a plausible other explanation for the occurrence of the AE.

Possibly

- The AE has a reasonable temporal relationship with drug administration.
- The AE may equally be explained by the study subject's clinically state, environmental or toxic factors, or concomitant therapy administered to the study subject.
- The relationship between study drug and AE may also be pharmacologically or clinically plausible.

Probably

- There is a reasonable temporal relation between the AE and the intake of the study medication, and plausible reasons point to a causal relation with the study medication.

Related

- Reasonable temporal relation between the AE and the intake of the study medication and
- There is no other explanation for the AE and

- Subsidence or disappearance of the AE on withdrawal of the study medication and
- Recurrence of the symptoms on restart at previous dose (only applies for re-institution of mediation).

Not assessable

- The causal relationship between the study drug and the AE cannot be judged.

9.6 Reporting procedures

A special section is designated to adverse events in the case report form. The following details must thereby be entered:

- Type of adverse event
- Start (date and time)
- End (date and time)
- Severity (mild, moderate, severe)
- Serious (no / yes)
- Unexpected (no / yes)
- Outcome (resolved, resolving, not resolved, resolved with sequelae, unknown, fatal)
- Relation to study drug (Related/ Probably/ Possibly/ Unlikely/ Not related/ Not assessable)

Adverse events will be documented in the case report form in accordance with the above mentioned criteria. Adverse events will subsequently be summarized in the development safety update report of the trial (9.6.3)

9.6.1 Reporting procedures for SAEs

In case of a serious adverse event, the Investigator has to use all supportive measures for best patient treatment. A written report is also to be prepared and provided to the sponsor immediately (at least within 24 hours).

should at least contain the following:

- Patient number
- Patient: sex
- The suspected investigational medical product (IMP)
- The adverse event assessed as serious
- Short description of the event and outcome

If applicable, the initial report should be followed by the Follow up report, indicating the outcome of the SAE. The sponsor will report safety information as required by CTR.

9.6.2 Reporting procedures for SUSAR

All SUSARs are reported by the sponsor via Eudravigilance (EVWeb) within 7 or 15 days, depending on the event (according to CTR), and will be included in the development safety update report (9.6.3)

Such reports shall be made by the sponsor and should content at least the following details:

- Patient number (study code/screening number)
- Patient: age in years, sex
- Name of Investigator and investigating site
- Period of administration
- The suspected investigational medical product (IMP)
- The adverse event assessed as serious and unexpected, and for which there is a **suspected** causal relationship to the IMP

- Concomitant disease and medication
- Short description of the event:
    - Description
    - Onset and if applicable, end
    - Therapeutic intervention
    - Causal relationship
    - Seriousness criteria or reportable reason

Electronic reporting should be the expected method for reporting of SUSARs to the competent authority. In that case, the format and content as defined by the regulatory requirements should be adhered to. The latest version of MedDRA should be applied. Lower level terms (LLT) should be used.

9.6.3 Development safety update report

A Development Safety Update Report (DSUR) will be provided by the Sponsor annually and submitted to the competent authorities and IEC via CTIS.

10 FOLLOW-UP

10.1 Follow-up of study participants including follow-up of adverse events

Two follow-up visits for clinical performance scores (CPC and mRS) and mortality are planned. All adverse events will be followed during the observation period, which lasts for 72h. The terminal elimination half-life of dexmedetomidine is approximately 1.5h. This means that theoretically after 6h this substance should be completely eliminated from the body. However, even if clearance of the drug is reduced by critical illness, therapeutic temperature management at 33 degrees or organ failure, this time span should suffice to observe any adverse effects triggered by the study drug.

Follow-up will be longer for serious or severe adverse events or for adverse events related to the study drug. As organ dysfunction and parameters indicative of organ injury are endpoints of this trial, they will also be followed-up beyond 72h.

This is a very sick patient population and we expect numerous adverse events.

10.2 Treatment after end of study

The treatment phase is short, because treatment aims at improving the post cardiac arrest syndrome. The observation period is 72h to assess safety and tolerability associated with the study drug. Patients are treated at the intensive care unit or at other specialized units beyond that point as part of routine of care. There is no specific treatment of patients planned beyond the 24h. That said, patients requiring dexmedetomidine as part of their routine treatment may of course receive it after the first 24h.

11 STATISTICAL METHODOLOGY AND ANALYSIS

11.1 Analysis sets

Two different analysis sets are defined

(Modified) Intention to treat set

This analysis set includes subjects who were randomized and in whom dexmedetomidine treatment was at least started.

Per-protocol set

This analysis set comprises all subjects who received study drug (for 24h) and did not violate the protocol in a way that might affect the evaluation of the effect of the study drug(s) on the primary objective, i.e., without major protocol violations.

11.2 Sample size considerations

In this trial, we will investigate the effects of dexmedetomidine on NSE values at 72 hours after cardiac arrest.

There are no data on the expected effects of dexmedetomidine on clinical outcomes after OHCA or on biomarkers of neurologic injury in humans. Although many publications address the performance of NSE as a biomarker for neurologic outcome, only a few report NSE as an outcome parameter in prospective, controlled trials with similar inclusion and exclusion criteria.

In a study by Nutma et al., using similar inclusion and exclusion criteria, NSE concentrations were 56 ± 13 µg/L (mean ± standard deviation [SD]) at 24 hours and 52 ± 14 µg/L at 72 hours after ROSC in the control group. These values are supported by data from a double-blind, randomized, placebo-controlled trial in swine, in which NSE concentrations in the placebo group reached 45 ± 8 µg/L after 24 hours, while they were 30 ± 8 µg/L in the low-dose group, which received 0.25 µg/kg/h dexmedetomidine, and 25 ± 5 µg/L in the high-dose group, which received 0.5 µg/kg/h.

In addition, we have analyzed unpublished data from the Vienna Cardiac Arrest Registry from 2019 to 2024. A total of 1204 cardiac arrest cases were treated at the Department of Emergency Medicine during that period. We aimed to use selection criteria that would allow analysis of a somewhat similar population (e.g., out-of-hospital cardiac arrest, time to ROSC of 10-60 minutes, cardiac and pulmonary cause of cardiac arrest, etc.). Of the remaining 268 cases, NSE values at 72h were available for only 116 (43%) patients. In the majority of the 152 missing 72h NSE values, the patient did not survive to 72h (n=86, 57%), making it impossible to measure NSE values. For the remaining missing values, one possible explanation is that the 48h values were very low, the patients' clinical course was favorable, and omitting NSE measurements was a deliberate choice. Another reason may be very high values at 48h, which also rendered the 72h value not very useful. To increase the sample size, we chose to impute NSE values at 48h when 72h values are unavailable, resulting in 160 (60%) patients. These assumptions yield a mean NSE of 58 ± 51 µg/L. The higher variability is not surprising, given that these patients were not included in a clinical trial with defined in- and exclusion criteria, but were collected consecutively. Furthermore, part of the variability may also be explained by data imputation. Because the trial will apply strict inclusion criteria and standardized sampling, we assumed a lower SD.

For the sample size calculation, we aimed to take these considerations into account and assumed a mean NSE concentration of 58 ± 40 µg/L in the control group and 38 ± 28 µg/L in the dexmedetomidine group 72 hours after ROSC. This corresponds to a 33% reduction in NSE concentrations, which is similar to the study in swine and to other clinical situations: e.g., in patients undergoing epilepsy surgery, dexmedetomidine reduced NSE after 24h to 13.5 ± 9.1 µg/L compared with 32.8 ± 43.4 µg/L in the control group. We have further assumed a two-sided alpha level of 5% and 80% power. This would result in a sample size of n=51 per group (calculated with a Wilcoxon- Mann-Whitney-U test using G.Power 3.1).

Given the uncertainty regarding the expected treatment effect, the high variability of NSE concentrations observed in institutional registry data, and possible missing data, we increased the target power to 85%. This resulted in a required sample size of 58 patients per group, which was rounded to 60 patients per group.

Relevant protocol deviations

All protocol deviations will be listed in the study report.

The investigator confirms that he will immediately report any event that may meet the definition of a serious breach to the sponsor according to the Regulation (EU) No 536/2014. This means any deviation of the approved protocol version or the clinical trial regulation that is likely to affect the safety, rights of trial participants and/or data reliability and robustness to a significant and relevant degree in a clinical trial.

The final classification of an event as a serious breach with subsequent notification within the regulatory timeline via CTIS will be done by the sponsor. The classification will be based on the "Guideline for the notification of serious breaches of Regulation (EU) No 536/2014" from the European Medicines Agency. Only those events that are relevant for the safety or physical or mental integrity, or the rights of one or more trial participants, that effect the scientific value of the trial, or that are serious and happened systematically will meet the classification criteria.

11.3 Statistical analysis plan

Baseline data and demographics will be analyzed using descriptive statistics. Continuous variables will be summarized using mean and standard deviation or median and interquartile ranges (IQR), and 5th and 95th percentiles, as applicable. Categorical variables will be summarized as counts and percentages. All statistical tests will use a two-sided alpha error of 5%.

The primary endpoint will be a ranked composite of death before 72 hours after ROSC and NSE concentration at 72 hours. Patients who die before 72 hours will be assigned the worst outcome rank, below all observed 72-hour NSE values among survivors. Among patients alive at 72 hours with available measurements, lower NSE concentrations will be considered more favorable and will be ranked accordingly. The sample size was estimated based on a clinically meaningful difference in NSE concentrations at 72 hours between treatment groups, informed by data from comparable populations. Assuming a 33% relative reduction in NSE concentrations at 72 hours in the dexmedetomidine group as compared with the placebo group, a two-sided alpha level of 0.05, and a power of 85%, the required sample size was 60 patients per group, calculated using a Mann-Whitney-U test for two independent groups.

The primary analysis will compare the distribution of the ranked endpoint between the dexmedetomidine and placebo groups using a Wilcoxon-Mann-Whitney test. Effect estimates will be expressed as the probability that a randomly selected patient in the dexmedetomidine group has a more favorable outcome than a randomly selected patient in the placebo group, with corresponding confidence intervals. Values >0.5 favor dexmedetomidine.

Although, missing data should be minimal, given that (i) all patients will be hospitalized, (ii) the active period of the trial is short, and (iii) we will assume highest ranks for those patients who die within the first three days of admission, we will conduct a sensitivity analysis in which 48h values will be substituted for 72h (last observation carried forward) in case of missing values.

As a supportive analysis NSE concentrations and all longitudinal biomarker endpoints of the trial will be analyzed using a linear mixed-effects model to account for the correlation of repeated measurements within individuals. The model will include treatment group (dexmedetomidine vs. placebo), time (modeled as categorical variable with levels corresponding to baseline, 6h, 12h, 24h, 48h, 72h), and the treatment-by-time interaction as fixed effects. The primary effect of interest is the treatment by time

interaction, which assesses whether the temporal evolution of NSE or other biomarkers differs between the treatment groups.

A subject-specific random intercept for each subject will be included to account for between-subject variability. If supported by the data, a random slope for time may additionally be incorporated to allow for subject-specific variation in trajectories.

Given the expected non-linear time course of the biomarkers of interest following resuscitation, time will be modeled as a categorical variable in the primary analysis, allowing for a flexible assessment of group differences at each clinically relevant time point without imposing assumptions about the functional form of the trajectory over time.

As a sensitivity analysis, time will additionally be modeled as a continuous variable using flexible functional forms (e.g., restricted cubic splines) to account for unequal spacing between measurements and to capture potential non-linear trends over time.

Model assumptions including approximate normality of residuals and homoscedascity of residual variance, will be assessed using graphical diagnostics. If required, appropriate transformations (e.g., logarithmic transformation of biomarkers concentrations) will be applied.

The linear mixed-effect modeling approach allows to use all available observations under the assumption that data are missing at random without the need for imputation of missing outcome measurements.

However, because death before 72 hours precludes measurement of NSE and may be related to disease severity, it will not be treated as missing data in the primary analysis but will instead be incorporated into the ranked composite endpoint. In the longitudinal analysis, all available NSE measurements obtained before death or last assessment will be included. This approach provides valid inference under a missing-at-random assumption for incomplete repeated measurements; however, because missingness due to death may be informative, results of the longitudinal analysis will be interpreted as supportive. Among patients who are alive at 72 hours, missing NSE values at that time point will be assumed to be missing at random, and analyses will be based on available outcome data without imputation. Sensitivity analyses will include evaluation of NSE concentrations at 72 hours among survivors and assessment of the robustness of the findings to alternative assumptions regarding missing data.

Further analyses may include comparison of biomarker concentrations at certain time points by the non-parametric Mann Whitney U test, as well as comparison of AUCs of biomarkers.

Clinical endpoints will be dichotomized into favorable and unfavorable neurological outcome (CPC 1-2 vs. CPC 3-5) and compared between the dexmedetomidine group and the placebo group using a Chi^2 test (or Fisher's exact test, as applicable) for the analysis at day 30 and at day 90. The same analysis will be performed for the comparison of the mRS (mRS 0-2 favorable outcome, mRS 3-6 unfavorable outcome), day 30 and 90 mortality, and occurrence of acute kidney injury or hypoxic hepatic injury within 72 hours of cardiac arrest. Mortality data will also be analyzed using time-to-event methods. Survival curves will be estimated using the Kaplan Meier method. Differences between treatment groups will be analyzed using the log-rank test. Furthermore, we will use Cox proportional hazard models, both univariate and multivariate (adjusted for traditional prognostication factors of CPR such as age, sex, time to sustained circulation, initial heart rhythm (shockable vs. non-shockable), MCS, etc.). Proportional hazards assumption will be checked.

All analyses will be conducted including all patients, in whom the interventional treatment was initiated ("Modified Intention-to-Treat"). In a second step, we will perform these analyses in the two subgroups: patients with and without eCPR. Furthermore, we will perform these analyses in the "per-protocol" population defined as those patients, who received the interventional treatment for the foreseen period without dose adjustments.

Dexmedetomidine pharmacokinetics and factors potentially influencing dosing will be assess using nonlinear mixed-effects modelling ('population pharmacokinetic modelling and simulations'). This approach allows to determine pharmacokinetic parameters characterizing the disposition and elimination of the drug (e.g., clearance, volume of distribution) for the typical ("mean") patient in the

population and subpopulations, as well as simultaneously for each individual patient, thus allowing to quantify and explain interindividual variability aside from residual unexplained variability. Furthermore, the developed model enables simulations to assess the adequacy of standard doses administered in the study and alternative, optimized dosing regimens for the critically ill population and subpopulations.

Quality-of-life outcomes (EQ-5D-5L index, EQ visual analogue scale, Hospital Anxiety and Depression Scale [HADS], Short Form-36, and Barthel Index) will be analyzed as exploratory endpoints. Continuous questionnaire scores will be analyzed using linear regression models with treatment group as the independent variable. If model assumptions are not met, non-parametric methods will be applied. Effect estimates with 95% confidence intervals will be reported. Because these analyses are exploratory, no adjustment for multiple comparisons will be performed.

Safety data will be presented using descriptive statistics.

11.4 Missing, unused and spurious data

We will not impute missing data. Obvious outliers or spurious data may be removed. This must be described in the final report.

Using the population pharmacokinetic model describing dexmedetomidine concentrations, even imbalanced and sparse data, e.g. incomplete concentration-time profiles, can be analysed. Concentration data below the limit of qualification will be handled depending on their proportion in the dataset (e.g. excluded from analysis or handled using likelihood methods (M3 method).

11.5 Endpoints analysis

11.5.1 Primary endpoint analysis

Ranked composite of death before 72h and NSE concentration at 72 h between the dexmedetomidine and the placebo period.

11.5.2 Secondary endpoint analysis

Secondary endpoints include

- NSE concentrations 72h after cardiac arrest between the dexmedetomidine and the placebo period
- NSE concentrations during the first 72h after cardiac arrest between the dexmedetomidine and the placebo period
- Ranked composite of death before 72h and S100ß concentration at 72h between the dexmedetomidine and the placebo period
- S100ß concentrations 72h after cardiac arrest between the dexmedetomidine and the placebo period
- S100ß concentrations during the first 72h after cardiac arrest between the dexmedetomidine and the placebo period
- C-reactive protein concentrations at 24h, 48h and 72h between the dexmedetomidine and the placebo period
- IL-6 concentrations during the first 72h after cardiac arrest between the dexmedetomidine and the placebo period

- Troponin T concentrations during the first 72h after cardiac arrest between the dexmedetomidine and the placebo period in patients with acute coronary syndromes as causative disease (i.e., those who require coronary interventions) up to 72h after cardiac arrest
- Occurrence of acute renal failure during the first 72h after cardiac arrest
- Occurrence of hypoxic hepatic injury during the first 72h after cardiac arrest

These biomarkers will be analyzed 72h after cardiac arrest. However, biomarker concentrations will also be quantified at baseline, 6h ± 2h , 12h ± 3h 24h ± 4h, 48h ± 6h and 72h ± 8h. The statistical analysis will incorporate these repeated measures within each subject

Pharmacokinetics: A population pharmacokinetic model will be calculated as explained above.

In vitro: impact of patient serum on endothelial permeability as well as the influence of dexmedetomidine on endothelial permeability using patient serum and established permeability increasing agents as stimuli.

11.5.3 Safety and tolerability endpoints

Safety data will be presented using descriptive statistics.

11.5.4 Baseline parameters and concomitant medications

Baseline parameters and concomitant medications will be tabulated. Continuous variables will be summarized using mean and standard deviation or median and interquartile ranges (IQR), as applicable. Categorical variables will be summarized as counts and percentages.

11.6 Interim analysis

Not applicable

11.7 Software program(s)

R, SPSS, GraphPad Prizm

12 DOCUMENTATION AND DATA MANAGEMENT

12.1 Documentation of study results

A subject screening and identification Log will be completed for all enrolled subjects with the reasons for exclusion.

12.1.1 Case report form (CRF)

Paper based work sheets will be used as source data. An eCRF will be programmed to store data electronically (ClinCase).

For each subject enrolled, regardless of study drug initiation, a CRF must be completed and signed by the Investigator or a designated sub-Investigator. This also applies to those subjects who fail to complete the study. If a subject withdraws from the study, the reason must be noted on the CRF. Case report forms are to be completed on an ongoing basis.

Screening failures should not be documented in the CRF, but in the Subject ID list and Enrolment log.

CRF entries and corrections will only be performed by study site staff, authorized by the Investigator.

In a paper based CRF all forms should be completed and must be legible. Entry errors have to be corrected according the ICH-GCP Guidelines.

The entries will be checked by trained personnel (Monitor) and any errors or inconsistencies will be checked immediately.

The monitor will collect original completed and signed CRFs at the end of the study. A copy of the completed and signed CRFs will remain on site (Medical University of Vienna is the sponsor of the trial).

Source data will be archived for 25 years. At the end of the trial electronic CRFs will be downloaded to a USB drive that will also be archived for that period.

Christian Schoergenhofer, the PI of the trial, will be the responsible Data Manager.

12.1.2 Data collection

Data collected at all visits are entered into an interactive form. The CRFs will be source documents verified following guidelines established before study onset as detailed in the Monitoring Plan. Maintenance of the study database will be performed by the study nurses that are part of the study team.

12.2 Safekeeping

The Investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified (according to ICH-GCP “essential documents”). These documents will be classified into two different categories: Investigator's study site file (ISF) with all essential documents regarding the study conduct, and subject clinical source documents.

The Investigator's file will contain all essential documents listed in ICH-GCP Guidelines section 8.

Subject clinical source documents include all patient hospital clinical records in original version, such as original laboratory reports, ECG, X-ray prints and other reports.

These two categories of documents must be kept on file by the Investigator for as long as needed to comply with the regulatory requirements, i.e. at least 25 years for the investigator’s file.

12.3 Quality control and quality assurance

12.3.1 Periodic Monitoring

The designated monitor will contact and visit the Investigator on a regularly basis and will be allowed to have direct access to all source documents needed to verify the entries in the CRFs and other protocol-related documents provided that subject confidentiality is maintained in agreement with local regulations. It will be the monitor's responsibility to inspect the CRFs at regular intervals according to the monitoring plan throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them.

Monitoring will be performed by the Koordinationszentrum für klinische Studien using a risk-based approach focusing on the primary endpoint, clinical performance scores, mortality and on safety and tolerability.

We plan to perform approximately monthly monitoring visits after a minimum of five new patients.

12.3.2 Audit and inspections

Upon request, the Investigator will make all study-related source data and records available to a qualified quality assurance auditor mandated by the sponsor or to competent authority inspectors. The main purposes of an audit or inspection are to confirm that the rights and welfare of the subjects have been

adequately protected, and that all data relevant for assessment of safety and efficacy of the investigational product have appropriately been reported to the sponsor.

12.4 Reporting and publication

12.4.1 Publication of study results

The findings of this study will be published by the sponsor (Investigators) in a scientific journal and presented at scientific meetings. The manuscript will be circulated to all authors before submission. Confidentiality of subjects in reports/publications will be guaranteed.

13 ETHICAL AND LEGAL ASPECTS

13.1 Informed consent of subjects

Following comprehensive instruction regarding the nature, significance, impact and risks of this clinical trial, the patient must give written consent to participation in the study.

During the instruction the trial participants are to be made aware of the fact that they can withdraw their consent – without giving reasons – at any time without their further medical care being influenced in any way.

In addition to the comprehensive instructions given to the trial participants by the Investigator, the trial participants also receive a written patient information sheet in comprehensible language, explaining the nature and purpose of the study and its progress.

The patients must agree to the possibility of study-related data being passed on to relevant authorities.

The patients must be informed in detail of their obligations in relation to the trial participants insurance in order not to jeopardize insurance cover.

13.2 Acknowledgement / approval of the study

The Investigator (or a designated CRO) will submit this protocol and any related document provided to the subject (such as subject information used to obtain informed consent) to an Ethics Committee (EC) and the Austrian Competent Authorities (Bundesamt für Sicherheit im Gesundheitswesen (BASG) represented by the Agency for Health and Food Safety (AGES Medizinmarktaufsicht) via CTIS. Approval must be obtained before starting the study.

The clinical trial shall be performed in full compliance with the legal regulations according to the Drug Law (AMG - Arzneimittelgesetz) of the Republic of Austria and the Regulation (EU) No 536/2014.

13.2.1 Changes in the conduct of the study

Protocol modifications

Proposed modifications must be submitted to the appropriate CA and ECs via CTIS. Substantial modifications may be implemented only after CA/EC approval has been obtained. Modifications that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving CA/EC approval. However, in this case, notification must be done as soon as possible after implementation.

Study Termination

If the sponsor or the Investigator decides to terminate the study before the planned completion, they will notify each other in writing stating the reasons of early termination. Both the sponsor and the

investigator will ensure the protection of the subjects' wellbeing. The sponsor will notify the regulatory authority as well as the ethics committee via CTIS about the premature termination. Documentation will be filed in the Trial Master File as well as in the Investigator Site File.

13.2.2 Results posting

Within one year after the final completion of the study, results posting will be done by the sponsor via CTIS according to Annex IV of the Regulation (EU) No 536/2014. Where the clinical trial protocol provides for an intermediate data analysis date prior to the end of the clinical trial, and the respective results of the clinical trial are available, a summary of those results shall be submitted via CTIS within one year of the intermediate data analysis date.

13.2.3 Notifications

The sponsor shall mandatory notify the start of a clinical trial, the first visit of the first subject, the end of the recruitment (or re-start of recruitment), the end of a clinical trial via CTIS according to the regulatory timelines as defined in the Regulation (EU) No 536/2014.

If applicable, the sponsor shall notify a temporary halt, when a temporarily halted clinical trial is resumed, an early termination, a serious breach, an unexpected event, an urgent safety measure and all inspection reports of third country authorities according to the regulatory timelines as defined in the Regulation.

13.3 Insurance

During their participation in the clinical trial the patients will be insured as defined by legal requirements. The Investigator of the clinical trial will receive a copy of the insurance conditions of the 'patients insurance'. The sponsor is providing insurance in order to indemnify (legal and financial coverage) the Investigator/center against claims arising from the study, except for claims that arise from malpractice and/or negligence. The compensation of the subject in the event of study-related injuries will comply with the applicable regulations.

Details on the existing patients insurance are given in the patient information sheet.

13.4 Confidentiality

The information contained in this document, especially unpublished data, is the property of the principal investigator and his co-authors. It is therefore provided to you in confidence as an Investigator, potential Investigator, or consultant, for review by you, your staff, and an Ethics Committee or Institutional Review Board. It is understood that this information will not be disclosed to others without written authorization from the principal investigator.

13.5 Ethics and good clinical practice (GCP)

The Investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" (as amended at the 75 WMA General Assembly, Helsinki, Finland, October 2024) and with the laws and regulations of the country in which the clinical research is conducted.

The Investigator of the clinical trial shall guarantee that only appropriately trained personnel will be involved in the study. All studies must follow the ICH GCP Guidelines and the regulatory requirements.

Therefore this study is conducted in compliance with the protocol, with the Regulation (EU) No 536/2014 and with the principles of good clinical practice.

14 REFERENCES

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15 Appendix I The EQ-5D-5L Questionnaire

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

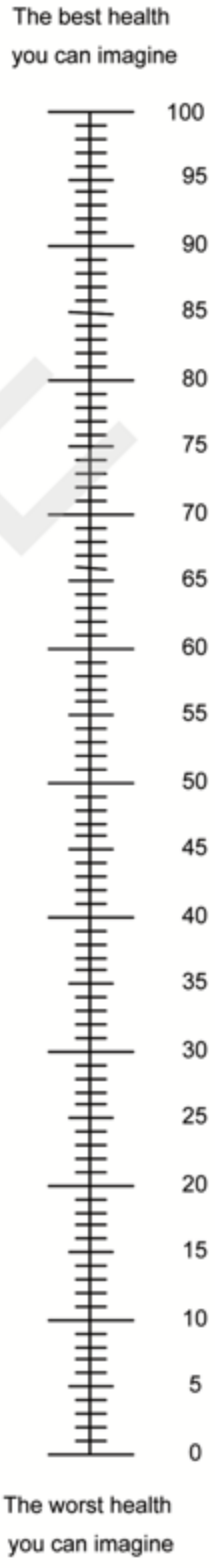
I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is **TODAY**.
- This scale is numbered from **0** to **100**.
- **100** means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an **X** on the scale to indicate how your health is **TODAY**.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



The EQ-5D-5L questionnaire (32).

16 Appendix II The Hospital Anxiety and Depression Scale

Hospital Anxiety and Depression Scale (HADS)

Tick the box beside the reply that is closest to how you have been feeling in the past week.
Don't take too long over you replies: your immediate is best.

D	A		D	A	
		I feel tense or 'wound up':			**I feel as if I am slowed down:**
	3	Most of the time	3		Nearly all the time
	2	A lot of the time	2		Very often
	1	From time to time, occasionally	1		Sometimes
	0	Not at all	0		Not at all
		I still enjoy the things I used to enjoy:			**I get a sort of frightened feeling like 'butterflies' in the stomach:**
0		Definitely as much		0	Not at all
1		Not quite so much		1	Occasionally
2		Only a little		2	Quite Often
3		Hardly at all		3	Very Often
		I get a sort of frightened feeling as if something awful is about to happen:			**I have lost interest in my appearance:**
	3	Very definitely and quite badly	3		Definitely
	2	Yes, but not too badly	2		I don't take as much care as I should
	1	A little, but it doesn't worry me	1		I may not take quite as much care
	0	Not at all	0		I take just as much care as ever
		I can laugh and see the funny side of things:			**I feel restless as I have to be on the move:**
0		As much as I always could		3	Very much indeed
1		Not quite so much now		2	Quite a lot
2		Definitely not so much now		1	Not very much
3		Not at all		0	Not at all
		Worrying thoughts go through my mind:			**I look forward with enjoyment to things:**
	3	A great deal of the time	0		As much as I ever did
	2	A lot of the time	1		Rather less than I used to
	1	From time to time, but not too often	2		Definitely less than I used to
	0	Only occasionally	3		Hardly at all
		I feel cheerful:			**I get sudden feelings of panic:**
3		Not at all		3	Very often indeed
2		Not often		2	Quite often
1		Sometimes		1	Not very often
0		Most of the time		0	Not at all
		I can sit at ease and feel relaxed:			**I can enjoy a good book or radio or TV program:**
	0	Definitely	0		Often
	1	Usually	1		Sometimes
	2	Not Often	2		Not often
	3	Not at all	3		Very seldom

Please check you have answered all the questions

Scoring:
Total score: Depression (D) _____ Anxiety (A) _____
0-7 = Normal
8-10 = Borderline abnormal (borderline case)
11-21 = Abnormal (case)

The Hospital Anxiety and Depression Scale (33).

17 Appendix III The Short-Form 36 Health Survey

SF-36 QUESTIONNAIRE

Name:_____ **Ref. Dr:**_____ **Date:** _____

ID#: _____ **Age:** _____ **Gender: M / F**

Please answer the 36 questions of the **Health Survey** completely, honestly, and without interruptions.

GENERAL HEALTH:
In general, would you say your health is:
Excellent Very Good Good Fair Poor

Compared to one year ago, how would you rate your health in general now?
Much better now than one year ago
Somewhat better now than one year ago
About the same
Somewhat worse now than one year ago
Much worse than one year ago

LIMITATIONS OF ACTIVITIES:
The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.
Yes, Limited a lot Yes, Limited a Little No, Not Limited at all

Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf
Yes, Limited a Lot Yes, Limited a Little No, Not Limited at all

Lifting or carrying groceries
Yes, Limited a Lot Yes, Limited a Little No, Not Limited at all

Climbing several flights of stairs
Yes, Limited a Lot Yes, Limited a Little No, Not Limited at all

Climbing one flight of stairs
Yes, Limited a Lot Yes, Limited a Little No, Not Limited at all

Bending, kneeling, or stooping
Yes, Limited a Lot Yes, Limited a Little No, Not Limited at all

Walking more than a mile
Yes, Limited a Lot Yes, Limited a Little No, Not Limited at all

Walking several blocks
Yes, Limited a Lot Yes, Limited a Little No, Not Limited at all

Walking one block
Yes, Limited a Lot Yes, Limited a Little No, Not Limited at all

Bathing or dressing yourself
◯Yes, Limited a Lot ◯Yes, Limited a Little ◯No, Not Limited at all

PHYSICAL HEALTH PROBLEMS:
During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

Cut down the amount of time you spent on work or other activities
◯Yes ◯No

Accomplished less than you would like
◯Yes ◯No

Were limited in the kind of work or other activities
◯Yes ◯No

Had difficulty performing the work or other activities (for example, it took extra effort)
◯Yes ◯No

EMOTIONAL HEALTH PROBLEMS:
During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

Cut down the amount of time you spent on work or other activities
◯Yes ◯No

Accomplished less than you would like
◯Yes ◯No

Didn't do work or other activities as carefully as usual
◯Yes ◯No

SOCIAL ACTIVITIES:
Emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

◯Not at all ◯Slightly ◯Moderately ◯Severe ◯Very Severe

PAIN:
How much bodily pain have you had during the past 4 weeks?

◯None ◯Very Mild ◯Mild ◯Moderate ◯Severe ◯Very Severe

During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

◯Not at all ◯A little bit ◯Moderately ◯Quite a bit ◯Extremely

Have you felt downhearted and blue?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

Did you feel worn out?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

Have you been a happy person?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

Did you feel tired?
- ◯ All of the time
- ◯ Most of the time
- ◯ A good Bit of the Time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

SOCIAL ACTIVITIES:
During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

- ◯ All of the time
- ◯ Most of the time
- ◯ Some of the time
- ◯ A little bit of the time
- ◯ None of the Time

ENERGY AND EMOTIONS:
These questions are about how you feel and how things have been with you during the last 4 weeks. For each question, please give the answer that comes closest to the way you have been feeling.

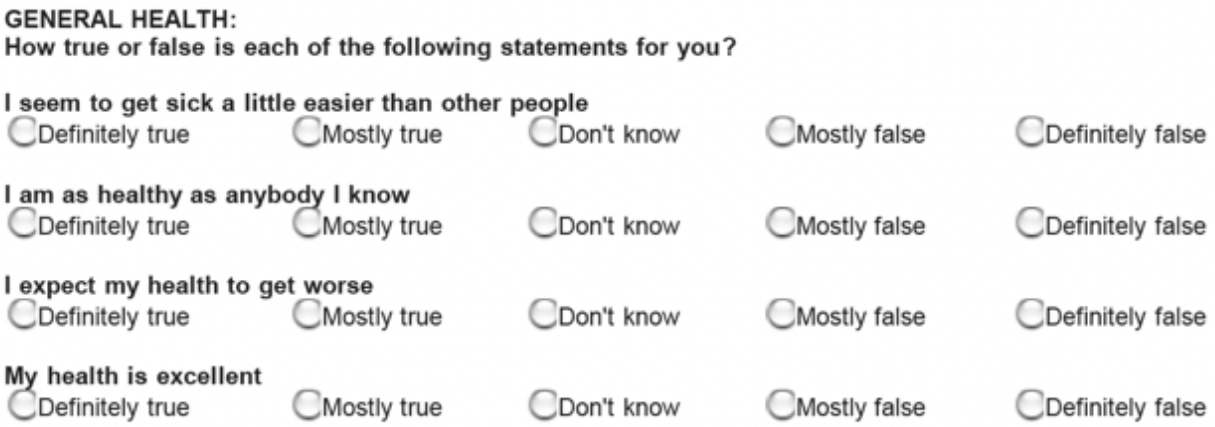
Did you feel full of pep?
- ○ All of the time
- ○ Most of the time
- ○ A good Bit of the Time
- ○ Some of the time
- ○ A little bit of the time
- ○ None of the Time

Have you been a very nervous person?
- ○ All of the time
- ○ Most of the time
- ○ A good Bit of the Time
- ○ Some of the time
- ○ A little bit of the time
- ○ None of the Time

Have you felt so down in the dumps that nothing could cheer you up?
- ○ All of the time
- ○ Most of the time
- ○ A good Bit of the Time
- ○ Some of the time
- ○ A little bit of the time
- ○ None of the Time

Have you felt calm and peaceful?
- ○ All of the time
- ○ Most of the time
- ○ A good Bit of the Time
- ○ Some of the time
- ○ A little bit of the time
- ○ None of the Time

Did you have a lot of energy?
- ○ All of the time
- ○ Most of the time
- ○ A good Bit of the Time
- ○ Some of the time
- ○ A little bit of the time
- ○ None of the Time



GENERAL HEALTH:
How true or false is each of the following statements for you?

I seem to get sick a little easier than other people
○ Definitely true ○ Mostly true ○ Don't know ○ Mostly false ○ Definitely false

I am as healthy as anybody I know
○ Definitely true ○ Mostly true ○ Don't know ○ Mostly false ○ Definitely false

I expect my health to get worse
○ Definitely true ○ Mostly true ○ Don't know ○ Mostly false ○ Definitely false

My health is excellent
○ Definitely true ○ Mostly true ○ Don't know ○ Mostly false ○ Definitely false

The Short-Form 36 Health Questionnaire (34)

18 Appendix IV Barthel Index

THE BARTHEL INDEX

Patient Name: _____
Rater Name: _____
Date: _____

Activity	Score
FEEDING 0 = unable 5 = needs help cutting, spreading butter, etc., or requires modified diet 10 = independent	_____
BATHING 0 = dependent 5 = independent (or in shower)	_____
GROOMING 0 = needs to help with personal care 5 = independent face/hair/teeth/shaving (implements provided)	_____
DRESSING 0 = dependent 5 = needs help but can do about half unaided 10 = independent (including buttons, zips, laces, etc.)	_____
BOWELS 0 = incontinent (or needs to be given enemas) 5 = occasional accident 10 = continent	_____
BLADDER 0 = incontinent, or catheterized and unable to manage alone 5 = occasional accident 10 = continent	_____
TOILET USE 0 = dependent 5 = needs some help, but can do something alone 10 = independent (on and off, dressing, wiping)	_____
TRANSFERS (BED TO CHAIR AND BACK) 0 = unable, no sitting balance 5 = major help (one or two people, physical), can sit 10 = minor help (verbal or physical) 15 = independent	_____
MOBILITY (ON LEVEL SURFACES) 0 = immobile or < 50 yards 5 = wheelchair independent, including corners, > 50 yards 10 = walks with help of one person (verbal or physical) > 50 yards 15 = independent (but may use any aid; for example, stick) > 50 yards	_____
STAIRS 0 = unable 5 = needs help (verbal, physical, carrying aid) 10 = independent	_____
TOTAL (0–100):	_____

The Barthel Index (35).