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Study protocoll of the prospective Study

Version 1.2

HaemoAdsorption Nach Reanimation An ECMO (HANRAE)

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Table of Contents

1. Principal Investigators‘ data

1.1 Name and Title of the study‘s principal investigator:

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2. Table of Abbreviations

ANOVA	analysis of variance
ASA	American Society of Anesthesiology
BNP	brain natriuretic peptide
CPR	cardiopulmonal resuscitation
CRP	C-reaktive protein
ECMO	extracorporal membrane oxygenation
GOT	Glutamate-Oxalacetate-Transaminase
GPT	Glutamate-Pyruvate-Transaminase
HR	heart rate
IL	Interleukin
MAP	mean arterial pressure
NSE	neuron-specific enolase
PCT	Procalcitonin
S1P	Sphingosin-1-Phosphate
TNF	tumor necrosis factor
CVP	central venous pressure

3. Purpose of the Study

A cardiac arrest with subsequent cardiopulmonary resuscitation (CPR) is even in case of a successful return of spontaneous circulation regularly associated with critical ischemia of body tissue. Together with damage caused by reperfusion, this is causing a systemic inflammatory reaction with release of cascades of pro- and antiinflammatory cytokines, taking part in impairing the clinical outcome of the patient.

Yet, for many patients it is even impossible to reconstitute a proper spontaneous circulation in the course of a mechanical and pharmaceutical CPR. Nowadays some of those, under certain circumstances, might be available for temporary support by veno-arterial extracorporal membrane oxygenation (va-ECMO)

To establish this device, a physican is installing central venous and arterial intravascular catheters during the course of the CPR. Those are then connected to an external system consisting of a pumping unit and a membrane for gas exchange, the oxygenator. This veno-arterial ECMO thus constitutes a modified version of a heart-lung-machine and is capable of performing a temporal and partial cardio-pulmonary bypass, relieving stressed heart and lung of the patient from a certain workload. The system‘s maintenance is performed by specifically trained physicians and technicians. It‘s modular concept allows for the exchange of certain parts with only minor or even entirely without interruptions in therapy. Thus, the additional implementation of a adsorber module as performed in this study, does not constitute a relevant threat to patient safety and survival.

As mentioned above, the concept of va-ECMO in resuscitated patients is to disburden vital organs in a critical phase and thus to facilitate their recovery under supportive intensive care and/or to get time to initiate further procedures in order to eliminate the original cause for cardiopulmonary arrest.

In this study, we aim to show, whether patients after CPR with subsequent va-ECMO profit from an additional modulation of the inflammatory reaction via hemoadsorption (Cytosorb®) in terms of levels of inflammatory cytokines and ultimaltely in organ function and survival.

4. Prior Knowledge

Patients with cardiopulmonary arrest and subsequent CPR are regularly harmed by a subsequent systemic inflammatory reaction with many parallels to those suffering from sepsis. In both groups, there is a significant increase in the levels of pro-inflammatory cytokines as interleukin 6 (IL-6). According to present data, there seems to be a positive correlation between the individual level of this increase and terminal mortality of the patients (1).

With hemoadsorption (Cytosorb®, MedaSorb Technologies, Princeton, N.J., USA), a device for effective elimination of cytokines like tumor necrosis factor α (TNF-α), interleukine 6 (IL-6) and 8 (IL-8) from the patient‘s bloodstream is available. This device is CE-certified for a use in extracorporal circulation and has been in clinical use worldwide for successful adsorbtion of cytokines, yet as to our knowledge never for routine elimination of circulating cytokines in a post-resuscitation setting so far.

Previous studies were able to establish the capability of the Cytosorb adsorber to eliminate IL-6, interleukine 10 and TNF-α from the serum and reduce the mortality under severe sepsis in an animal model (2).

As for the clinical application in humans, there are several case reports attributing a positive outcome for severely affected patients to the use of hemoadsorption. A significant reduction of cytokines by the device has been shown in patients under sepsis (4), systemic inflammation caused by sepsis (5) and systemic inflammation without sepsis (6) (own publication, *submitted*).

As to our knowledge, there is currently no data available for the application of this device in severe systemic inflammation after cardiac arrest, CPR or va-ECMO subsequent to the named conditions.

5. Study Conception and Project Outline

5.1 primary and secondary endpoints

In this study, we aim to show that hemoadsorption (Cytosorb®) is a viable option to reduce serum levels of proinflammatory cytokines in patients under va-ECMO after CPR. Specifically, our hypothesis constitutes significantly lower levels of IL-6 and TNF-α in patients undergoing hemoadsorption for 48 hours compared to a control group receiving standard medical care at va-ECMO during a 72 hours-timeframe.

Primary outcome parameters

Laboratory parameters expressing an inflammatory response constitute the primary outcome of our study. Thus,

IL-6, TNF-α, S1P, PCT, CRP, leucozytes

are to be measured at time points 0,6,12,24,48 and 72 hours after administration to va-ECMO.

Secondary outcome parameters

Those are supposed to reflect secondary and tertiary effects of the hemoadsorption on organ function and overall survival of the patients:

1. Laboratory and clinical parameters of inflammation and organ damage/dysfunction (cardiovasculary, pulmonary, hepatic, renal, neuronal):

    - NSE, (free) hemoglobine, haptoglobine, BNP, Troponine, Creatinekinase, Myoglobin, GOT, GPT, Creatinine
    - cardiovasculary: (MAP, CVP, HR,. echocardiographic parameters)
    - pulmonary: oxygenation/decarboxylation, acid-base-balance by blood gas analysis

2. Parameters of treatment:
    - Cardiovasculary: Dependence on inotropics, and vasopressors, volume substitution or elimination,
    - pulmonary: relevant parameters of ventilation
    - additional organ replacement therapy (dialysis, hepatic replacement)

3. Parameters of terminal outcome
    - mortality within the first 30 days after inclusion into the study
    - length of stay in intensive care/in hospital

5.2 Principal study desing

The study is single-centered, prospective, controlled and randomized.

5.3 Sample size

We expect a difference of 20-30% in serum concentration of IL-6 at 24 hours after inclusion (about 60µg/L difference at total levels of 200-300µg/L, standard deviation 90µg/L). With 18 patients per group, the study could determine a significant difference between the groups with a power of 90% and an error propability of 5% by ANOVA.
To attribute for post-hoc exclusion or drop-out of participants with an estimated rate of 10%, we suggest a sample size of 20 patients per group, adding to a total of 40 participants in the study

This calculation is based on the publication of Adrie C. Et al. (1) and on a previous study performed in our department (publication pending).

5.4 Criteria for In- and Exclusion

As our hypothesis proposes a relevant effect on any person undergoing treatment by va-ECMO post CPR, the criteria for in- and exclusion of study participants are in accordance with the clinical indication for this procedure as specified in the standard operating procedures of the University Cardiac Arrest Center Hamburg:

Indications:

- Age: 18 to 75 years
- cardiopulmonary arrest with
  - a potentially reversible cause
  - an initially observed rhythm qualifying for defibrillation and
  - sufficient CPR prior to va-ECMO

relative contraindications:

- severely lowered pH and/or severely increased lactate in arterial blood gas analysis at the time of evaluation
- protracted CPR for >30 minutes prior to evaluation for va-ECMO

absolute contraindications:

- Do-not-resuscitate (DNR)-order stated by the patient prior to current event
- context of palliative care
- severe persisting neurological impairment, proven or highly likely at the time of evaluation for va-ECMO
- severe trauma
- relevant acute hemmorhagic event
- presence of comorbidities with severe reduction of life expectancy (like, but not limited to chronic pulmonary disease, chronic heart failure prior to event, hepatic cirrhosis, malignoma)

5.5 Informed consent

Due to the fact, that the patients are regularly expected to be unconscious at the time of evaluation for va-ECMO and thus for inclusion in our study and any delay e.g. for consultation of a legal representative is not feasible in an emergency context, the primary inclusion into the study goes in accordance with the clinical criteria stated above. A retrospective informed consent has to be sought at the earliest possible moment from a legal representative or the patient itself, should the latter regain sufficient consciousness. In case of secondary disapproval and thus, drop-out from the study, samples and data gathered up to that time are handled in accordance with state and federal German law. This procedure has specifically been evaluated and approved by the responsible ethics committee.

5.6 Randomization

Randomization is performed at the time of establishment of va-ECMO. Drawing without returning of prepared sealed envelopes will ensure an identical size of 20 participants each for control and intervention groups .

5.7 Clinical anaethesiologic and intensive Care of the Participants

The general anaethesiologic and intensive care is not affected by inclusion into this study. All procedures are conducted in accordance with therapeutic standards of our hospital, referencing best medical practice and applicable guidelines.

The sample size to analyze the cytokine levels is estimated to amount to 5ml per time point.

5.7. Statistical analysis

Statistical analysis is conducted using the software package SPSS. To characterize the patients, we are going to conduct descriptive analytics in dependence on the statistical distribution of variables.

Continuous variables like hemodynamic and laboratory parameters are analyzed using a multifactorial ANOVA or a mixed lineary model.

Nominal or categorized variables are going to be analyzed in four-to-multi-square-tables with chi-square-tests or Fisher‘s exact test.

5.8. **Time points and parameters**

Time points:

1) on administration to va-ECMO
2) 6 hours post administration to va-ECMO
3) 12 hours post administration to va-ECMO
4) 24 hours post administration to va-ECMO
5) 48 hours post administration to va-ECMO
6) 72 hours post administration to va-ECMO

Parameters

1. inflammatory:
    - IL-6
    - TNFα
    - S1P
    - PCT
    - CRP
    - leukozytes

2. cardiocirculatory
    - MAP, CVP, HR
    - Dependence on external inotropes or vasopressors
    - BNP, Troponine, Creatinekinase, Myoglobine, Haptoglobine
    - echocardiographic parameters

3. gas exchange in arterial blood gas analysis
    - partial pressures of Oxygen and Carbon dioxide
    - pH and lactate
    - Base excess

4. hepatic
    - GOT, GPT

5. renal
    - diuresis
    - fluid balance
    - serum creatinine
    - glomerularic filtration rate estimated by CKD-EPI-formula

Additionally, we are going to evaluate demographic data (functional status in accordance with ASA-classification, age, sex, size, total body weight) and periprocedural parameters with specific focus on CPR (duration/time prior to va-ECMO, cause of arrest).

Overview of the study

Principal investigator	Prof. Dr. Jens Kubitz
Title	HaemoAdsorption Nach Reanimation An ECMO (HANRAE)
Hypothesis	Additional hemoadsorption (Cytosorb®) in the extracorporal circulation established by va-ECMO in patients after cardiac arrest and CPR reduces the levels of IL-6 and TNFα in the serum.
Target population	Patients under va-ECMO subsequent to cardiac arrest and CPR
Primary endpoints	Levels of IL-6 und TNFα in the patients‘ serum in an interval of 72 hours post resuscitation
Secondary endpoints	• Laboratory and clinical parameters of inflammation and organ damage/dysfunction (cardiovascular, pulmonary, hepatic, renal, neuronal) • Parameters of intensive care treatment (e.g. ventilation, additional organ replacement therapy) • Parameters of terminal outcome (mortality)
study design	single-centerd, prospective, controlled, randomized
inclusion criteria	• Age ≥ 18 years • cardiac arrest with CPR • application of va-ECMO for insufficient spontaneous circulation
exclusion criteria	• contraindication for va-ECMO in accordance with the standard operating procedures of the University Cardiac Arrest Center Hamburg *ECMO bei Reanimation – see 5.4 for details*
sample size	We expect a difference of 20-30% in serum concentration of IL-6 at 24 hours after inclusion (about 60µg/L difference at total levels of 200-300µg/L, standard deviation 90µg/L). With 18 patients per group, the study could determine a significant difference between the groups with a power of 90% and an error of propability of 5% by ANOVA. To attribute for post-hoc exclusion or drop-out of participants, we suggest a sample size of 20 patients per group, adding to a total of 40 participants in the study.
randomization	Randomization is performed as drawing without returning to ensure an identical size for control and intervention groups.

Hamburg, 29.12.2017

6. Literature

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