Official Study Title:

Endotoxin and Cytokine Adsorption Properties of the Oxiris Hemofilter in Septic Shock:

Evaluation of Saturation Phenomena and Inflammatory Mediators' Clearance Capacity

Sponsor:

Pauls Stradiņš Clinical University Hospital, Riga, Latvia

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Investigator-Initiated Study

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Study Title: Endotoxin and cytokine adsorption properties of the Oxiris hemofilter in septic shock: evaluation of saturation phenomena and inflammatory mediators' clearance capacity.

Brief Title: OxiCLEAR (Oxiris Cytokines and Endotoxin Adsorption Rate) study: Evaluating Saturation Phenomena of Oxiris Membrane

Hypothesis: We hypothesize that the Oxiris membrane becomes saturated with endotoxin and inflammatory mediators over time (24-hours period), resulting in reduced adsorptive capacity and potentially limiting the therapeutic efficacy of blood purification in patients with septic shock.

Objectives:

[O1] to assess the dynamics of the adsorption capacity of AN69-polyethylenimine membrane (Oxiris®) for endotoxin over a 24-hour period in septic shock patients who are admitted to the intensive care unit.

[O2] to assess the dynamics of the adsorption capacity of AN69-polyethylenimine membrane (Oxiris®) for pro-and anti-inflammatory mediators over a 24-hour period in septic shock patients who are admitted to the intensive care unit.

[O3] To assess the association between decreased performance of the AN69-polyethylenimine membrane (Oxiris®), potentially related to early saturation, and the clinical course and 28-day mortality in patients with septic shock.

Primary Endpoint:

The primary endpoint of this study **is to evaluate the adsorption capacity** of the AN69-polyethylenimine membrane (specifically the Oxiris® hemofilter) for endotoxins **[O1]** and inflammatory mediators **[O2]** in patients with septic shock undergoing continuous veno-venous hemofiltration (CVVH). Objective **[O2]** will be extended to include the evaluation of cytokine removal through convective transport by analyzing cytokine concentrations in the effluent fluid. This will allow for a more accurate assessment of the membrane's net adsorption capacity by distinguishing between cytokine clearance via convection and adsorption. The study aims to assess the correlation between inlet endotoxin concentration and the rate of adsorption over a continuous 24-hour period, in order to characterize membrane saturation dynamics and their potential clinical implications **[O3]**.

Number of Sites: Pauls Stradinš Clinical University Hospital (PSCUH), Department of Anesthesiology and Intensive care, Riga, Latvia.

Study Population:

The study population will include adult patients (≥18 years of age) of any gender who are diagnosed with septic shock and admitted to the intensive care unit (ICU). All enrolled patients must receive continuous veno-venous hemofiltration (CVVH) with a high-adsorptive AN69-polyethyleneimine membrane (Oxiris®) for a minimum duration of 24 hours as part of their clinical management.

Key Inclusion Criteria: Should meet all the criteria mentioned below:

1. Septic shock patient with a Dynamic scoring system[1] of 6-8 points

/Septic shock definition based on Sepsis-3: a vasopressor requirement to maintain a mean arterial pressure of 65 mmHg or greater and serum lactate greater than 2mmol/L despite optimal (30ml/kg body weight) bolus fluid resuscitation/

2. CVVH with AN69-polyethylenimine membrane for at least 24 h

3. Age >18 years
4. Informed consent provided by the patient or by a person with decisional responsibility.

1. Kogelmann, K.; Hübner, T.; Schwameis, F.; Drüner, M.; Scheller, M.; Jarczak, D. First Evaluation of a New Dynamic Scoring System Intended to Support Prescription of Adjuvant CytoSorb Hemoadsorption Therapy in Patients with Septic Shock. J. Clin. Med. ***2021****, 10, 2939. https:// doi.org/10.3390/jcm10132939*

Key Exclusion Criteria:

Patients will be excluded from the study if they meet any of the following conditions: age under 18 years, pregnancy, known contraindications to citrate anticoagulation, or a clinical judgment indicating a high likelihood of death within the next 24 hours due to irreversible comorbidities (e.g., end-stage cardiac, pulmonary, or hepatic disease; hepatorenal syndrome; or advanced, uncontrolled malignancy).

Time of follow-up / Duration of subject participation: Each subject will be followed for a total duration of 28 days after the initiation of CVVH therapy with the Oxiris® hemofilter. Data collection will include intensive monitoring during the first 24 hours to assess adsorption dynamics, followed by ongoing clinical assessments up to day 28 to evaluate outcomes such as organ function, treatment response, and the potential impact of membrane saturation.

Modality of CRRT: to ensure uniformity of therapy across all study participants, standardized CRRT settings will be applied in accordance with clinical best practices. All patients enrolled in the study and undergoing CRRT will be treated using continuous veno-venous hemofiltration (CVVH) with the PrisMax or Prismflex system and the high-adsorptive AN69-polyethyleneimine membrane (Oxiris®). CRRT parameters will follow the institution's local protocol for septic shock patients and include:

- Anticoagulation: Continuous citrate infusion for circuit anticoagulation
- Filtration dose: 25–30 mL/kg/h
- Blood flow rate: 100–150 mL/min
- Net ultrafiltration rate: 0 mL/h (as the standard approach for hypovolemic septic shock patients)
- Replacement fluid rate: 1000–1500 mL/hour, with a 50/50 distribution between pre-dilution and post-dilution

These standardized parameters are intended to minimize variability and ensure consistent treatment conditions for accurate comparison of adsorption dynamics and patient outcomes.

Data collection:

Sample Collection Time Points: blood samples for endotoxin and inflammatory mediators measurement will be collected at the following time points: **T0 (Baseline):** Immediately before initiation of CVVH with Oxiris® and during the next 24 hours of the treatment at the following times: 1hour (T 1), 3hours (T 3), 6hours (T 6), 12hours (T 12) and 24 hours (T 24).

Sample Collection Sites in the CRRT circuit:

- **Inlet / Arterial Line (C_in** for endotoxin and cytokines
- **Outlet / Venous Line (C_out)** for endotoxin and cytokines
- **Effluent Samples:** Effluent fluid samples will be collected at the corresponding time points to detect anti- and pro-inflammatory mediators, enabling assessment of clearance via the convection mechanism.

Sample Processing: immediately after collection, blood samples will be centrifuged for 10 minutes, and the plasma and samples from effluent will be stored at **–80°C** until analyses will be performed for endotoxin and inflammatory mediators concentration is performed.

Endotoxin concentrations will be measured using assays based on *LSBio, Gram Negative Endotoxin (Competitive EIA) ELISA Kit* with detection range of 100-2500 pg/ml and sensitivity for endotoxin concentration detection of ≤0.005 EU/ml. Method Precision (Analysis of Preliminary Data): Across all sample series and dilution levels, the relative standard deviation (RSD) ranged from 2.0% to 2.3%, indicating low variability and high precision of the method under the tested conditions.

Inflammatory mediators' concentrations in the plasma and effluent will be measured using the following methods:

- Inflammatory mediator concentrations will be assessed using the **Multiplex Biochip Analyzer Evidence Investigator** platform (Randox Laboratories, EV3513 Cytokine and growth factors array), integrated within the RADOX system. The platform enables simultaneous quantification of multiple cytokines from a single sample using **Cytokine Array I**, ensuring high sensitivity, reproducibility, and broad biomarker coverage relevant to inflammatory response profiling.
- **Cytokines Included in the Cytokine Array I panel: EGF** (Epidermal Growth Factor), **IFN-γ** (Interferon gamma), **IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10**, **MCP-1** (Monocyte Chemoattractant Protein-1), **TNF-α** (Tumor Necrosis Factor alpha), **VEGF** (Vascular Endothelial Growth Factor).

Investigators will ensure strict adherence to the study protocol. All anonymized patient medical records will be securely stored in electronic format and retained indefinitely to allow for future review, audit, or verification, if required.

To assess the efficacy of the Oxiris® hemofilter in adsorbing endotoxin **[O1]** and inflammatory mediators **[O2]**, and to evaluate the impact of premature membrane saturation on the clinical course and outcomes **[O3]** of septic shock patients, the following data will be collected during the course of this study:

- patient data (age, gender, BMI); main diagnosis prompting ICU admission, pre-existing comorbidities, severity as assessed using Sequential Organ Failure Assessment (SOFA) and Dynamic score system, source control interventions, and bacterial growth in blood. Antibacterial therapy (dosage and administration of a/b therapy (intermittent vs continuous) and timing of initiation of antibiotic therapy.

- To detect endotoxin **[O1]** and inflammatory mediators **[O2]** absorption capacity of Oxiris® membrane, and **the membrane saturation limit**, endotoxin and inflammatory mediators concentration will be measured on both sides of the filter,in addition to inflammatory mediators detection in the effluent Blood and effluent samples will be obtained from each patient immediately before initiation of the treatment with Oxiris® hemofilter (T0), and during the next 24 hours of the treatment at the following times: 1h (T 1), 3h (T 3), 6h (T 6), 12h (T 12) and 24 hours (T 24). The **adsorption rate** will be calculated based on the concentration difference between inlet and outlet samples, combined with the blood flow rate. These values will be expressed as units per minute (U/min) and normalized to the membrane surface area (U/cm²/min) to enable comparison across patients and time points. Inflammatory mediators concentrations will be determined by measuring levels in both blood and effluent samples, accounting for clearance through the filter. Effluent clearance of each cytokine will be calculated by multiplying its concentration in the effluent by the effluent flow rate.

- Clinical outcomes **[O3]** will be defined by various measures, including 28-day mortality, renal recovery, need for further renal support, length of ICU stay, length of in-hospital stay and vasopressor-free days. Secondary outcomes also include hemofilter efficacy markers (at 24h), such as hemodynamic improvement, a decrease in lactate levels, reduced need for vasopressors, and a decline in inflammatory markers like Procalcitonin (PCT) and C-reactive protein (CRP).

- Laboratory parameters will be measured at baseline (T0) and at T24 (the end of the first Oxiris® session) to assess systemic response and organ function. The evaluated parameters will include full blood count (hemoglobin, leukocyte count, thrombocyte count), coagulation markers (D-dimers, prothrombin time (PT), international normalized ratio (INR)), inflammatory markers (procalcitonin (PCT), C-reactive protein (CRP)), kidney and liver function biomarkers (urea, creatinine, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), bilirubin), as well as nutritional and electrolyte levels (albumin, phosphorus (P), magnesium (Mg)), as recommended by standard clinical practice for patients undergoing CRRT.

- Respiratory and hemodynamic parameters, including heart rate, arterial blood pressure, central venous pressure, and vasopressor dosage, will be recorded in the ICU in accordance with the standard monitoring protocol. Arterial blood gas analysis will be performed every 8 hours during the first 24 hours of treatment.

CRRT parameters, including blood flow rate, replacement fluid rates, and transmembrane pressure (TMP) and Δ within the CRRT circuit, will be documented to assess filter lifespan and the occurrence of premature filter clotting or clogging. Additionally, the rate of complications potentially related to CVVH therapy will be recorded

Statistical Methods - Primary Endpoint Analysis:

The primary endpoint of this study is the **adsorption rate** of endotoxins and inflammatory mediators by the Oxiris® hemofilter, defined as the difference between inlet and outlet concentrations (in U/mL), multiplied by blood flow rate (mL/min). This value reflects the amount of adsorptive molecules removed per minute and is further normalized to the filter's surface area (U/cm²/min).

To evaluate the primary objective, the statistical analysis will test the correlation between inlet concentration and adsorption rate across multiple time points per patient over a 24-hour treatment period.

Analytical Approach:

- Pearson correlation coefficients will be calculated between inlet concentration and the corresponding adsorption rate at each available time point (excluding T0 to account for system equilibration).
- Each individual correlation coefficient will be transformed using Fisher's Z transformation to stabilize variance.
- The mean Fisher Z value across all patients and time points will be computed and subsequently back-transformed to obtain the overall correlation coefficient (ρ).
- A one-sided hypothesis test will be used to test:
    - Null Hypothesis (H_0): ρ = 0.5
    - Alternative Hypothesis (H_1): ρ = 0.8
- Statistical significance will be assessed at α = 0.05.

Sample Size Justification:

A one-tailed test is justified because the study specifically expects a decrease in the correlation between inlet endotoxin concentration and adsorption rate as membrane saturation occurs. Since membrane saturation can only reduce adsorption efficiency—not increase it—testing for a decrease (rather than any change) aligns with the biological mechanism and improves the study's statistical power. Using standard formulas for correlation testing with Fisher Z-transformation, a minimal sample size of **24 patients** is required to detect a correlation of 0.8 versus 0.5 with 80% power and α = 0.05 (one-tailed). These calculations are reproducible and may be verified using standard statistical software (e.g., G*Power, R, or STATA). However, a target of **29 patients** was chosen to provide a buffer for attrition and variability, maintaining statistical power and strengthening the validity of the study outcomes.

Software and Tools: statistical analyses will be performed using R (v4.3 or later) and validated using GraphPad Prism or SPSS, with full scripts and datasets available for reproducibility.

In addition, the project will utilize **MATLAB** and **SAS** for specialized analysis needs:

- **MATLAB** (MathWorks) will be used for advanced mathematical modeling, including time-dependent adsorption kinetics, membrane saturation dynamics, and nonlinear regression.
- **SAS** (Statistical Analysis System) will be employed for confirmatory statistical modeling and clinical data management.

Statistical Methods - Secondary Endpoint(s) Analysis:

Secondary outcomes will include hemofilter efficacy markers at 24 hours, specifically hemodynamic improvement (e.g., increase in mean arterial pressure, decrease in heart rate), reduction in lactate levels, decreased vasopressor requirements, and a decline in inflammatory markers such as procalcitonin (PCT) and C-reactive protein (CRP).

- Continuous variables will be reported as mean ± standard deviation or median with interquartile range, depending on data distribution. Categorical variables will be summarized as counts and percentages. Between-group comparisons will be performed using the Student's t-test or Mann-Whitney U test for continuous variables and the chi-square or Fisher's exact test for categorical variables. Changes in continuous secondary outcomes over 24 hours will be assessed using paired t-tests or Wilcoxon signed-rank tests, as appropriate.
- Missing data will be handled using complete case analysis; if the proportion of missing data exceeds 5%, multiple imputation techniques will be considered. Statistical significance will be set at a two-sided p-value < 0.05. Based on preliminary estimates and accounting for the inclusion of hemofilter adsorption rate as a covariate, a total sample size of 29 patients is planned to ensure sufficient power to detect meaningful differences in primary outcomes.

Ethics:

The study has been approved by the Ethics Committee as part of a PhD project entitled *'Endotoxin and cytokine adsorption properties of the AN69-based polyethyleneimine membrane in septic shock patients.'* The approval was granted under decision number 2-PĒK-4/429/2022 on September 29, 2022