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Clinical paper

Endothelial activation/injury and associations with severity of post-cardiac arrest syndrome and mortality after out-of-hospital cardiac arrest☆

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ABSTRACT

Background: Post-cardiac arrest syndrome (PCAS) is characterized by whole-body ischemia triggering systemic inflammation and damage of the endothelium. This study investigated the relationship between systemic inflammation, endothelial damage and severity of PCAS and the association between endothelial damage and outcome after out-of-hospital cardiac arrest (OHCA).

Methods: In this post hoc study, we analyzed 163 comatose patients included at a single center in the target temperature management (TTM) trial, randomly assigned to TTM at 33 °C or 36 °C for 24 h. Endothelial biomarkers (syndecan-1, thrombomodulin, sE-selectin, sVE-cadherin) and the inflammatory biomarker interleukin-6 (IL-6) were measured at admission (baseline) and 24, 48 and 72 h after OHCA. Severity of PCAS was assessed by Sequential Organ Failure Assessment score. Mortality at 30-days was evaluated by Cox regression analysis.

Results: By linear regression, baseline IL-6 levels (two-fold) was independently associated with glycocalyx damage (syndecan-1 (10.3 ng/ml ($p = 0.01$))), endothelial activation (sE-selectin (2.0 ng/ml ($p = 0.03$))) and endothelial damage (thrombomodulin 0.7 ng/ml ($p = 0.0005$)) at 24 h after OHCA. Adjusted for baseline IL-6, a two-fold increase in thrombomodulin from baseline to 48 h (1.7 (0.9–2.4), $p < 0.0001$) and 72 h (1.5 (0.6–2.3), $p < 0.0007$) was more closely associated with severity of PCAS than IL-6. Levels of syndecan-1, thrombomodulin and sVE-cadherin was not influenced by level of target temperature but levels of sE-selectin was significantly lower in the 36 °C group (−55 ng/ml (95%CI: −53 to −58 ng/ml), $p = 0.005$) compared to the 33 °C group. High levels of thrombomodulin at 24 h (HR = 2.1 (1.3–3.3), $p = 0.001$) and 48 h (HR = 1.75 (1.0–2.8), $p = 0.02$) were associated with increased 30-day mortality in univariate analysis, but not in multivariable analyses.

Conclusion: In comatose survivors after OHCA treated with TTM, systemic inflammation was associated with endothelial activation and endothelial damage. Sustained endothelial damage was independently associated with severity of PCAS, adjusted for level of systemic inflammation. TTM at 36 °C compared to 33 °C after OHCA was associated with lower endothelial activation, but not endothelial damage.

Clinical Trial Registration: URL: clinicaltrials.gov/ct2/show/NCT01020916. Unique identifier: NCT01020916

Introduction

Development of the post-cardiac arrest syndrome (PCAS) is closely linked to exposure of whole-body ischemia-reperfusion during cardiac arrest and after return of spontaneous circulation (ROSC) often leading to multi-organ dysfunction.[1] Despite great emphasis on post-resuscitation care and focus on strategies to

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prevent secondary injury to vital organs, survivors after out-of-hospital cardiac arrest (OHCA) still carry a severe prognosis with high risk of subsequent mortality and morbidity.[1–3] The vascular endothelium represents the largest organ system exposed to the ischemia-reperfusion injury inducing endothelial activation and endothelial glycocalyx damage causing increased vascular permeability, promotes interaction between endothelium and leukocytes and endothelial mediated inflammation.[4–6] PCAS is characterized by microcirculatory dysfunction, endothelium mediated leukocyte adhesion resembling a sepsis-like syndrome and severity of PCAS has previously been associated with high levels of systemic inflammation.[7–10] Excessive sympathoadrenal activation is associated with endothelial damage and outcome after cardiac arrest, but level of systemic inflammation may also play an important role in development of endothelial activation and damage.[11] However, our understanding of the pathophysiology in PCAS, the interactions between inflammatory systems, endothelial damage and organ injury is sparse, limiting our capability to develop future treatment strategies. Whether interventions aiming to protect or restore endothelial functions may be beneficial in limiting organ injury or improving outcome is unknown.

The objective of the present explorative study was to investigate the relation between systemic inflammation, endothelial damage and severity of PCAS evaluated by serial measurements of endothelial markers, hypothesizing the sustained endothelial damage was associated with increased severity of PCAS. Furthermore, to investigate the effect of target temperature on endothelial damage and to report the possible prognostic value of endothelial damage on outcome in OHCA patients.

Methods

This study was a post hoc analysis from a single center (Rigshospitalet, Copenhagen) including patients participating in the prospective investigator-initiated, multi-center, randomized, parallel-group, and assessor-blinded target temperature management (TTM) trial (ClinicalTrials.gov number NCT01020916).[12] Patients were randomized in a 1:1 fashion to targeted temperature management at 33 °C (TTM33) or 36 °C (TTM36) for 24 h after cardiac arrest. The TTM protocol was approved by the Ethics Committee of the Capital Region Copenhagen (H-1-2010-059) and by the Danish Data Protection Agency. Written informed consent was obtained from patients' next of kin and general practitioner in all cases and/or from patients regaining consciousness after cardiac arrest. In brief, inclusion criteria were adult patients (≥18 years) resuscitated from OHCA of presumed cardiac cause, who remained unconscious (Glasgow Coma Score (GCS) <8) for more than 20 min after sustained return of spontaneous circulation (ROSC). Main exclusion criteria included unwitnessed asystole, a body temperature of less than 30 °C and severe shock at time of admission to hospital defined as sustained systolic blood pressure less than 80 mmHg despite administration of fluids, vasopressors, inotropes and/or treatment with intra-aortic balloon pump or left ventricular assist device.[13]

Pre-hospital data regarding the cardiac arrest including initial rhythm, witnessed arrest, administration of bystander cardiopulmonary resuscitation (CPR) and time to ROSC were systematically collected according to Utstein guidelines.[14]

Eligible patients for the present study were screened consecutively and included at Rigshospitalet, Copenhagen University Hospital, Denmark (in total $n = 171$). In this post hoc study, we analyzed available baseline and follow-up blood samples from patients included in a previously published baseline study ($n = 163$) reporting the prognostic value of baseline sympathoadrenal activation and endothelial damage after OHCA.[11] This study aimed to explore the PCAS syndrome focusing on the dynamic change in endothelial function within the first 72 h post arrest and the relationship with systemic inflammation evaluated by serial blood samples reflecting endothelial activation and damage.

Patient management

All patients were admitted to the intensive care unit (ICU) for advanced post-cardiac arrest care and were sedated, intubated and mechanically ventilated throughout the 36-h intervention period according to the study protocol.[13] The sedation/analgesic protocol included administration of propofol and fentanyl titrated to achieve a Richmond Agitation-Sedation Scale (RASS) score of −4 and if necessary a bolus of a neuromuscular blocking agent was administered to reduce shivering. All patients were actively cooled with the use of a surface cooling device (Thermowrap® with Allon® unit, MTRE, Israel). Active cooling was initiated immediately after randomization with an induction period of 4 h to achieve the target temperature, followed by a maintenance period of 24 h with subsequent active rewarming of no more than 0.5 °C per hour to 37 °C in both groups. According to the TTM-protocol, all patients received active treatment for a minimum of 72 h after the rewarming phase of the target temperature intervention.[13]

Crystalloid fluids were administered in all patients with general treatment goals for central venous pressure (CVP) of 10–15 mmHg to optimize right heart filling pressure, mean arterial pressure (MAP) ≥65 mmHg to secure adequate organ perfusion and urine output >1.5 ml/kg/h. Inotropics/vasopressors were used to achieve adequate organ perfusion primary directed by clinical parameters as MAP, CVP and urine output to achieve the pre-defined hemodynamic treatment goals.

Severity of PCAS was assessed daily by the Sequential Organ Failure Assessment (SOFA) score.[15] These data along with demographics, medical history, characteristic of the cardiac arrest and survival status assessed by the study end date in July 2013 was available from the TTM database.

Blood samples and enzyme linked immunosorbent assay (ELISA) analyses

Blood was sampled from an arterial line with first blood samples (T0 = baseline) obtained approximately 5 min after inclusion and randomization in the TTM-study in the majority of patients. The following serial blood samples were collected at 24, 48 and 72 h after OHCA. Lactate levels were analyzed immediate after sampling from the arterial line, whereas plasma samples were centrifuged at 4 °C at 3000 rpm for 10 min within a half hour of sample collection. EDTA-plasma and serum aliquots were stored in Nunc™ tubes (Nunc, Roskilde, Denmark) at −80 °C, until all samples were collected for analysis.

Biomarkers of endothelial glycocalyx damage (syndecan-1),[16] endothelial cell activation (sE-selectin),[17,18] endothelial cell injury (soluble thrombomodulin)[17–19] and endothelial junction disruption (sVE-cadherin)[20] were measured in uniplicate by commercially available immunoassays in serum according to the manufactures recommendations: syndecan-1 (Diaclone, Nordic Biosite, Copenhagen, Denmark; lowest limit of detection (LLD) 4.94 ng/ml, intra- and inter-assay coefficient of variation (CV) were 6.2% and 10.2%); sE-selectin (R&D Systems Europe, Ltd., Abingdon, UK; LLD 0.009 ng/ml, intra- and inter-assay CV were 5.1–6.9% and 7.3–8.6%); thrombomodulin (Diaclone, Nordic Biosite, Copenhagen, Denmark; LLD 0.31 ng/ml, intra- and inter-assay CV were 3.9% and 9.8%) and sVE-cadherin (R&D Systems Europe, Ltd., Abingdon, UK; LLD 0.113 ng/ml, intra- and inter-assay CV were 3.1–5.1% and 4.4–7.2%). Activation of the inflammatory system was assessed in EDTA plasma by measurement of the pro-inflammatory cytokine