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Clinical paper

Complement activation is associated with poor outcome after out-of-hospital cardiac arrest

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Abstract

Background: Cardiopulmonary resuscitation after cardiac arrest initiates a whole-body ischemia-reperfusion injury, which may activate the innate immune system, including the complement system. We hypothesized that complement activation and subsequent release of soluble endothelial activation markers were associated with cerebral outcome including death.

Methods: Outcome was assessed at six months and defined by cerebral performance category scale (1–2; good outcome, 3–5; poor outcome including death) in 232 resuscitated out-of-hospital cardiac arrest patients. Plasma samples obtained at admission and day three were analysed for complement activation products C3bc, the soluble terminal complement complex (sC5b-9), and soluble CD14. Endothelial cell activation was measured by soluble markers syndecan-1, sE-selectin, thrombomodulin, and vascular cell adhesion molecule.

Results: Forty-nine percent of the patients had good outcome. C3bc and sC5b-9 were significantly higher at admission compared to day three ($p < 0.001$ for both) and in patients with poor compared to good outcome ($p = 0.03$ and $p < 0.001$, respectively). Unadjusted, higher sC5b-9 at admission was associated with poor outcome (odds ratio 1.08 (95% CI 1.01–1.14), $p = 0.024$). Adjusted, sC5b-9 was still associated with outcome, but the association became non-significant when time to return-of-spontaneous-circulation above 25 min was included as a covariate. Endothelial cell activation markers increased from admission to day three, but only sE-selectin and thrombomodulin were significantly higher in patients with poor versus good outcome ($p = 0.004$ and $p = 0.03$, respectively) and correlated to sCD14 and sC5b-9/C3bc, respectively.

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Conclusion: Complement system activation, reflected by sC5b-9 at admission, leading to subsequent endothelial cell activation, was associated with poor outcome in out-of-hospital cardiac arrest patients.
Keywords: Out-of-hospital cardiac arrest, Outcome, SC5b-9 protein complex, Cardiopulmonary resuscitation, Return of spontaneous circulation, Immune system, Endothelial cells

Introduction

Out-of-hospital cardiac arrest (OHCA) remains an important public health matter with a high mortality rate and is the third leading cause of death in Europe.[1] After successful resuscitation, the main reason for mortality and morbidity is the duration of hypoxia and a whole-body ischemia/reperfusion injury.[2] The brain is most susceptible to this reperfusion injury,[3] and two-thirds of hospital deaths are due to the neurological injury,[4]

The pathophysiology of ischemia/reperfusion injury is very complex with a plethora of players. Cardiopulmonary resuscitation (CPR) initiated reperfusion triggers the innate immune system by danger agents acting as ligands for pattern recognition molecules of the various branches of the innate immunity, including the complement system.[5,6] This induces a secondary and broad-acting systemic inflammatory response which, when over-activated or dysregulated, leads to tissue damage, organ failure and in worst case to death.[7]

Complement is present in plasma and thus immediately activated upon injury through the classical, lectin, or alternative pathway. All three pathways converge at the level of C3, which gets cleaved and forms a protease that cleaves C5 leading to the formation of the terminal complement complex. The terminal complement complex is either incorporated in cellular membranes, where it may result in lysis and inflammation, or it is released as a soluble molecule (sC5b-9) to the fluid phase.[8] All C3 and C5 derived complement activation products, including the anaphylatoxins C3a and C5a, can activate endothelial cells.

The aim of the present study was to investigate if complement activation with subsequent endothelial cell activation as part of the initial ischemia/reperfusion injury was associated with poor cerebral outcome and death in patients resuscitated after OHCA.

Material and methods

The present study is a planned sub-study of the prospective observational Norwegian Cardio-Respiratory Arrest Study (NORCAST, NCT01239420).[9] The aim of NORCAST was to assess the ability of currently recommended diagnostic tools to identify patients with a poor prognosis. Importantly, results of prognostic tests were blinded to clinicians to avoid hasty withdrawal decisions and self-fulfilling prophecies. The design and patient population in NORCAST has been previously described in detail.[9] Briefly, 259 comatose adult OHCA patients admitted to Oslo University Hospital Ullevål were included between October 1st, 2010 and January 30th, 2014. Post-resuscitation care was performed according to local standard procedures including targeted temperature management to 33 °C (TTM33) for 24 h and immediate coronary angiography with subsequent percutaneous coronary intervention, if a cardiac cause was suspected.[9] Hypoxic cause of cardiac arrest was defined as a non-cardiac cause of the arrest, where hypoxia of different reasons resulted in unresponsiveness, absence of breathing and loss of heart function. The Sequential Organ Failure Assessment (SOFA score) was performed to assess the extent of a patient's organ failure within the first 24 h of admission. Neurological outcome at 6 months was assessed by Neurology specialists who were blinded to all clinical and paraclinical scores during hospital admission and well acquainted with Cerebral Performance Category (CPC, 1–2 good outcome, 3–5 poor outcome with 5 including death) scoring. The examination on which CPC scoring was based consisted of structured neurological and cognitive examination as well as queries of functional level to patients and next-of-kin. For complement activation markers, the upper reference limit for each assay was set to the 95th percentile of a healthy reference population consisting of 20 female and 20 male blood donors without cardiovascular or autoimmune diseases.[10] Upper reference limit of sC5b-9 is >0.7 complement activation units (CAU)/mL and for C3bc >9 CAU/mL.[10] For endothelial markers such international reference limit do not exist and venous EDTA-blood obtained from twelve healthy volunteers with a close to similar age (median 59 years, interquartile range 51–68 years) and gender distribution (7 female: 5 male) compared to the study population was used as control.

Blood sampling protocol

At admission (before initiation of TTM33) and after 72 h (after rewarming), peripheral venous blood was obtained in ethylenediaminetetraacetic acid (EDTA) vacutainer tubes (BD, Plymouth, UK). Samples were immediately kept on crushed iced and centrifuged within 30 min at 2500 g for 20 min at 4 °C. Plasma was collected and stored at −80 °C until analyses.

Complement activation markers

Complement activation products, C3bc (common for classical, lectin and alternative pathways) and sC5b-9 (soluble terminal complement complex), were measured by in-house enzyme-linked immunosorbent assays (ELISA) which has previously been described[11,12] and later modified according to the protocol presented in Ref. 10.

Endothelial activation markers and sCD14

Soluble endothelial activation markers syndecan-1, sE-selectin (sCD62E), thrombomodulin (sTM), vascular cell adhesion molecule 1(sVCAM-1) and sCD14 were measured by DuoSet ELISA (R&D Systems, Minneapolis, MN) in EDTA plasma.

Ethics and data management

The Regional Committee for Medical Research Ethics of South-Eastern Norway approved NORCAST (Approval number REK S-O/A - 2010/1116a). Ethics and study management have been previously reported.[9] Written informed consent was obtained from close relatives or guardians within 24 h after hospitalization and from all patients regaining consciousness and decision-making capacity within six

months. Clinical, biochemical and outcome data were prospectively collected from medical records, questionnaires, and paramedic records as presented in the Utstein criteria.[13] Blood samples from healthy donors were collected into a biobank approved by the Regional Ethical Committee (REK S-04114).

Statistical analysis

Data were presented as medians, 25th–75th percentile, or frequencies and percentages, as appropriate. Patients with good and poor outcome were compared by Mann–Whitney *U* test for continuous and χ^2-test or Fisher's exact test for categorical data. The difference in complement or endothelial activation between CPC groups was assessed by Mann–Whitney *U* test. Kruskal–Wallis test was used for comparison of more than two groups. Bonferroni correction was applied to correct for multiple testing. The Wilcoxon signed-rank paired test was used to compare the complement and endothelial activation at admission and day three. Associations between cerebral outcome and SOFA score, time from cardiac arrest-to-return-of-spontaneous circulation (time-to-ROSC), cardiac arrest (unwitnessed vs. witnessed), initial rhythm (shockable vs. non-shockable), bystander CPR (yes vs. no), and cause of cardiac arrest were assessed by unadjusted and adjusted (multivariable) logistic regression model. To assess whether sC5b-9 and C3bc at admission was associated with the odds for poor outcome separately as well as combined, unadjusted logistic regression models were estimated. The models were further adjusted for SOFA score, time-to-ROSC, and cause of cardiac arrest. Interactions between sC5b-9 and time-to-ROSC, and between C3bc and time-to-ROSC were included into the adjusted model if significant. The results of logistic regression models were presented as odds ratios (ORs) with the corresponding 95% confidence intervals (CIs) and p-values. The regression coefficients and standard errors (SE) were presented for the variables included into the interaction term. For easier interpretation, the interactions were illustrated graphically. To assess how well the sC5b-9 and C3bc distinguishes between those with good and poor outcome, the area under the characteristic (ROC) curve with the corresponding 95% CI was calculated. The logistic regression models were estimated to assess the association between the CPC score and sC5b-9 at admission adjusted for endothelial markers Syndecan-1, E-selectin, Thrombomodulin, and VCAM, and CD14 measured at day three, one at a time. Also, the model combining all endothelial markers and CD14 was estimated. The models were adjusted for SOFA score, time-to-return-of-spontaneous circulation (time-to-ROSC), and cause of cardiac arrest. Area under the ROC curve calculated for the unadjusted and each adjusted model was compared. Correlation between complement and endothelial activation markers was assessed using Spearman's rank test. Results with *p*-values <0.05 were regarded as significant. Statistical analyses were performed in Statistical Package for the Social Sciences (SPSS) software (version 26, IBM, Armonk, NY), Stata (version 16.0, StataCorp, College Station, TX), SAS (version 9.4, SAS Institute, Cary, NC) and GraphPad Prism (version 8, GraphPad Software, San Diego, CA).

Results

Out of 259 successfully resuscitated patients after OHCA, 232 were included into the study (Fig. 1). Median age was 63 (54–69), 83% were male, and 82% had cardiac cause of arrest (Table 1). Outcome

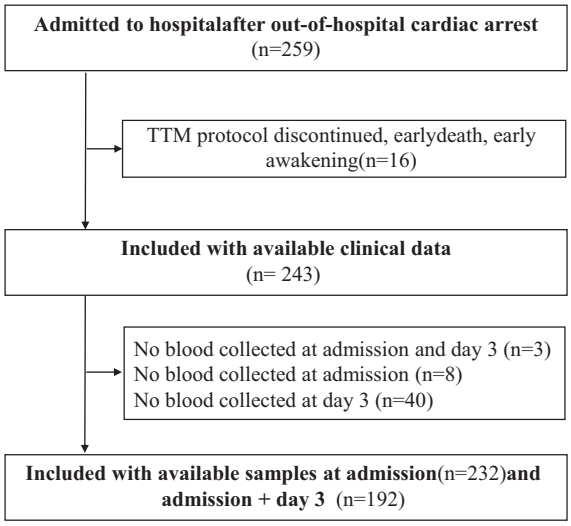

Fig. 1 – Flow chart of patient population in this study. Enrolment in study and exclusions, availability of clinical data and blood samples at each time point. TTM; target temperature management.

defined as best CPC score within six months after OHCA, was poor for 49% and good for 51% of patients with significant differences in time-to-ROSC and SOFA score at admission (Table 1). Clinical variables significantly associated with poor outcome in adjusted logistic regression analysis were non-shockable initial cardiac arrest rhythm, longer time-to-ROSC and higher overall organ dysfunction during the first 24 h after admission (Suppl. Table 1). Unwitnessed cardiac arrest was associated with outcome in unadjusted logistic regression model only, while performance of bystander CPR was not significantly associated with outcome (Suppl. Table 1).

Complement activation products

C3bc and sC5b-9 were markedly higher compared to upper reference limit in all patients (n = 232) both at admission and day three (Fig. 2A, B). C3bc and sC5b-9 were both significantly higher at admission compared to day three. Patients with poor outcome had significantly higher levels of C3bc and sC5b-9 at admission compared to patients with good cerebral outcome (Fig. 2A, B).

In unadjusted models, higher sC5b-9 at admission was associated with poor outcome (OR 1.08, 95% CI (1.01–1.14), $p = 0.02$, Suppl. Table 2), while C3bc was not (Suppl. Table 3). The area under the ROC curve (AUC) was 0.65 95% CI (0.57; 0.72) for sC5b-9 and 0.57 (0.49; 0.65) for C3bc. There was no additive effect on the ROC curve by combining sC5b-9 and C3bc (AUC 0.66 (0.58; 0.73)). Longer time-to-ROSC and higher SOFA scores at admission were also associated with poor outcome (OR 1.06 (1.05–1.09), $p < 0.001$ and OR 1.23 (1.06–1.43), $p = 0.006$, respectively, Suppl. Table 2). Interaction between sC5b-9 and time-to-ROSC was significant in an adjusted model ($p = 0.015$, Suppl. Table 2). Post hoc analysis of the interaction term showed that odds for poor outcome were increasing with increasing values of sC5b-9 when time-to-ROSC was below 30 min, while odds for poor outcome were dependent on time-to-ROSC alone at 30 min and longer (Suppl. Table 2, Fig. 3). There was no significant interaction between C3bc and time-to-ROSC in an unadjusted model,

Table 1 – Demographics and clinical characteristics at admission.

	Total (n = 232)	Good outcome (CPC 1–2) (n = 118)	Poor outcome (CPC 3–5) (n = 114)	*p*-value
Age (years)	63 (54–70)	62 (54–69)	64 (55–71)	*0.3*[a]
Sex				*0.5*[b]
Female	39 (17)	14 (36)	25 (64)	
Male	193 (83)	104 (54)	89 (46)	
Cause of cardiac arrest (CA)				
Acute myocardial infarction	89 (38)	48(54)	41 (46)	*0.5*[b]
Chronic cardiac disease	71 (31)	40 (56)	31 (44)	*0.3*[b]
Arrythmia (VF)	29 (13)	18 (62)	11 (38)	*0.2*[b]
Hypoxia induced CA	31 (13)	9 (29)	22 (71)	***0.01***[b]
Other causes	5 (2)	2 (40)	3(60)	*0.7*[c]
Unknown	7 (3)	1 (14)	6 (86)	*0.6*[c]
Survivors	123 (53)	114 (93)	9 (7)	<***0.001***[b]
Scores at admission				
SOFA score	11 (9.5–12)	10 (9–12)	11 (10–12)	<***0.001***[a]
Time-to-ROSC (min)	25 (16–33)	19 (12–28)	30 (24–40)	<***0.001***[a]

Data presented as median (25th–75th percentile) or n (%). Cerebral outcome is best CPC within 6 months after cardiac arrest. CPC; cerebral performance category, VF; ventricular fibrillation, CA; cardiac arrest, SOFA; Sequential Organ Failure Assessment, Time-to-ROSC; time to return of spontaneous circulation, min; minutes.

[a] Mann–Whitney *U* test.

[b] χ^2-test or

[c] Fisher's exact test.

and C3bc alone was not associated with outcome (Suppl. Table 3). Hypoxia as the cause of cardiac arrest increased the odds for poor outcome, with increased levels of sC5b-9 and C3bc at admission in comparison to acute myocardial infarction (OR 6.78 (1.90–24.16), $p = 0.003$, and OR 5.97 (1.75–20.36), $p = 0.004$, respectively, Suppl. Tables 2 and 3).

sCD14

sCD14, a co-receptor of TLR, was significantly higher in cardiac arrest patients (n = 192) compared to healthy controls and significantly higher at day three compared to admission (Suppl. Fig. 1). However, sCD14 was not associated with outcome alone or in adjusted models (Suppl. Table 4).

Markers of endothelial activation and damage

Glycocalyx damage marker sSyndecan-1 was higher in OHCA patients (n = 192) compared to healthy controls at admission and day three (Fig. 4A). It was significantly higher at admission compared to day three in OHCA patients (Fig. 4A). Markers of endothelial activation sE-selectin, sVCAM-1 and sTM were consistently higher in OHCA patients and concentrations at day three were significantly increased compared to admission (Fig. 4B–D). Among the four markers, only sE-selectin and sTM were significantly higher in patients with poor outcome compared to patients with good outcome (Fig. 5A, B). C3bc correlated significantly with sSyndecan-1 and sTM at admission ($R = 0.26$, $p < 0.001$ and $R=0.17$, $p = 0.02$, respectively) and day three ($R = 0.19$, $p = 0.007$ and $R=0.2$, $p = 0.007$, respectively, Suppl. Fig. 2). Likewise, sC5b-9 correlated significantly with sSyndecan-1 and sTM at admission ($R = 0.25$, $p < 0.001$ and $R=0.23$, $p = 0.001$, respectively) and at day three ($R=0.16$, $p = 0.03$ and $R = 0.23$, $p = 0.002$, Suppl. Fig. 2). sCD14 was significantly correlated with sE-selectin at day three ($R = 0.44$, $p < 0.001$). No other important correlations were observed (Suppl. Fig. 2). sE-selectin was the only endothelial parameter significantly associated with outcome in an unadjusted model (OR 1.02 [1.00–1.05], $p = 0.038$), but did not improve the discriminative ability of the model with only sC5b-9 (Suppl. Table 4). Adjustment did not improve the discriminative ability of sC5b-9 in combination with endothelial markers except for E-selectin with additive effect on the ROC curve when sC5b-9 was combined with sE-selectin (AUC 0.79 (0.72–0.85), $p = 0.047$, Suppl. Tables 4 and 5).

Discussion

A whole-body inflammatory reaction follows resuscitation after OHCA. In the present study, we show that initial strong activation of the complement system, in particular reflected by the terminal pathway activation product sC5b-9, was associated with poor long-term outcome. Following the initial rapid complement activation, endothelial activation occurred after admission. While the endothelial damage marker Syndecan-1 peaked at admission, all other measured markers of endothelial activation showed highest level at day three in contrast to complement activation, which then had markedly declined.

Complement activation

Complement activation at admission was associated with cerebral outcome. Complement has been shown to get activated immediately after initiation of resuscitation in OHCA patients with a swift decline after ROSC.[14] This might be due to release of damage associated molecular patterns (DAMPs) from injured cells followed by a chain of injurious events[15] leading to activation of complement signalling pathways, in particular the lectin pathway.[16] While the aforementioned study showed no relation of lectin pathway activation and outcome, this study confirms that the end-product of complement system activation sC5b-9 is associated with poor outcome. TTM33

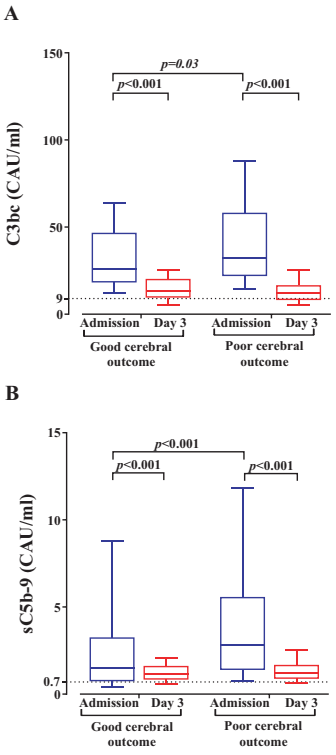

Fig. 2 – Patients (n = 232) with successful resuscitation after out-of-hospital-cardiac-arrest. Plasma levels of C3bc and sC5b-9 at admission (blue lines) were above upper reference limit (line) in most of the patients, significantly higher at admission compared to day three (red lines) and higher in patients with poor compared to good cerebral outcome (A, B).
Cerebral outcome was defined by cerebral performance category (CPC) as good; CPC 1–2 and poor; CPC 3–5. Data is shown as box plots with median as line and box indicating 25th–75th percentile and whiskers representing 10th–90th percentiles. Wilcoxon signed-rank paired test and non-paired Mann–Whitney *U* test.
CAU; complement arbitrary unit.

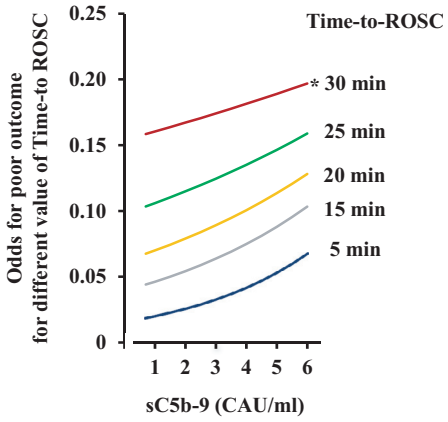

Fig. 3 – Interaction between sC5b-9 and time-to-ROSC is associated with poor outcome. Increasing values of sC5b-9 were associated with poor outcome when time-to-ROSC was ≤25 min but not when time-to-ROSC was 30 min or higher (asterix). Odd ratios adjusted for covariates SOFA, Time-to-ROSC and cause of cardiac arrest. CAU; complement arbitrary unit, Time-to-ROSC; time-to-return-of-spontaneous-circulation, SOFA; Sequential Organ Failure Assessment.

treatment of OHCA patients leads to suppression of complement activation with return to levels above reference limit after rewarming[17] while one study reports that TTM33 lead to reduction of the regulatory protein Map19 of the complement lectin pathway compared to TTM36.[18] All patients in this study were treated with TTM33 and it might thus be speculated that the initial complement activation is of special importance for outcome in OHCA patients. In line with this are findings that the degree of complement activation correlates with the severity of heart failure in patients developing cardiogenic shock after acute myocardial infarction.[19] Furthermore, blockade of C5 reduced infarct size and improved cardiac function in an experimental porcine model of severe myocardial infarction.[20]

An important factor influencing the reperfusion injury and degree of neurological injury is the duration of hypoxia i.e., the time-to-ROSC.[21] As in previous studies, we also verified an association between time-to-ROSC and poor cerebral outcome.[22] We add that for the same time-to-ROSC, odds for poor outcome increased with increasing levels of sC5b-9, suggesting that increased complement activation per se had an effect on outcome and might be an effect-modifier. The effect of association between sC5b-9 and outcome was absent in patients with time-to-ROSC 30 min, indicating that the impact of inflammation on cerebral outcome may be reduced when time-to-ROSC is long and ischemic brain damage becomes the only decisive factor. Despite the association between sC5b-9 and poor outcome, complement activation cannot be used as a decisive prognostic marker, given the large inter-patient variations of absolute values, which were in this study almost all above an internationally accepted upper reference limit.[10] However, complement inhibition in OHCA patients might be explored in further studies as inhibition of C5 has been shown to reduce ischemia/reperfusion injury in general, and our data on sC5b-9 support this hypothesis.

sCD14

sCD14 is a multifunctional molecule, which recognises and binds endogenous and exogenous danger signals.[23] It is also a co-factor for several TLRs which are mainly expressed by monocytes and macrophages. In the present study, sCD14 was increased upon admission and highest on day three, suggesting a maintained release, which might be due to shedding of membrane-bound CD14 protein or secretion via intracellular vesicles by inflammatory cells.[24] The prolonged release of sCD14 may indicate prolonged activation of the immune system which is in agreement with the pattern of complement and endothelial activation.

Endothelial activation

The endothelium is an active component of innate immunity. It gets injured during ischemia/reperfusion injury,[25] e.g. by complement. This again leads to retrograde complement activation resulting in a vicious circle with exaggerated inflammatory reaction causing tissue damage and organ failure.[26]

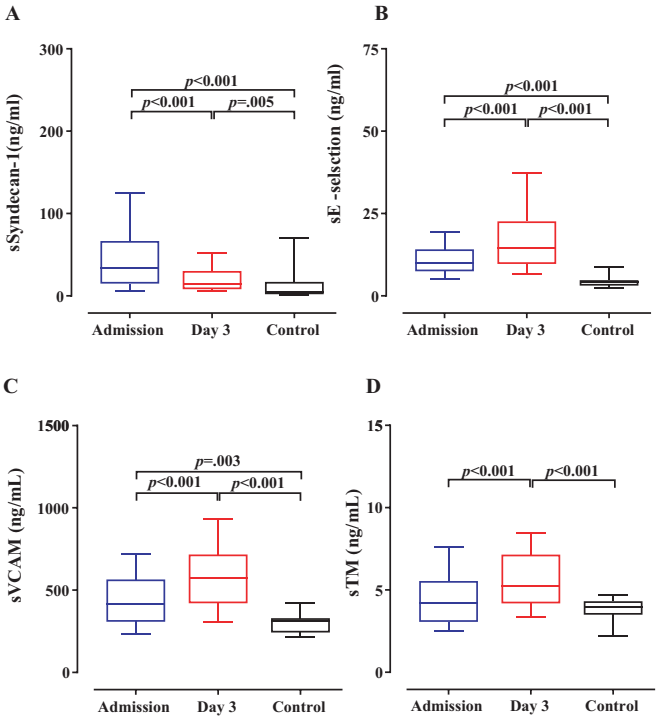

Fig. 4 – Patients (n = 192) with successful resuscitation after out-of-hospital-cardiac-arrest and blood samples both at admission and day three. Plasma levels of sSyndecan-1 (A), sE-selectin (B), and sVCAM (C) were significantly higher compared to healthy controls (black lines) at admission (blue lines) and day three (red lines), while sTM (D) was significantly higher at day three, only. sSyndecan was significantly higher at admission compared to day three, while sE-selectin, sVCAM and sTM were higher at day three compared to admission. Data is shown as box plots with median as line and box indicating 25th–75th percentile and whiskers representing 10th–90th percentiles. Wilcoxon signed-rank paired test and non-paired Mann–Whitney *U* test. sTM; sThrombomodulin.

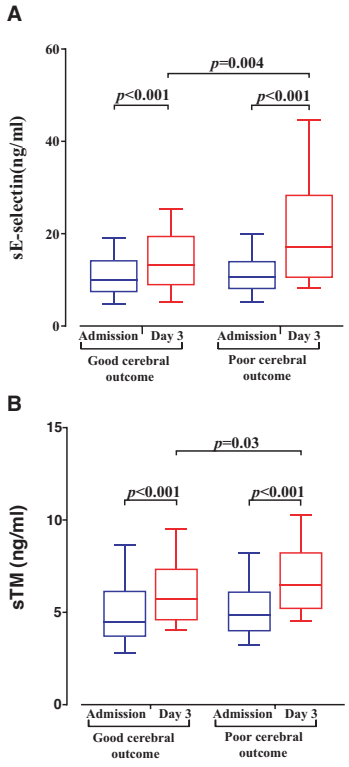

Fig. 5 – sE-Selectin (A) and sTM (B) in patients (n = 192) with successful resuscitation after out-of-hospital-cardiac-arrest. In patients with poor cerebral outcome, sE-selectin and sTM were significantly higher at day three (red lines) compared to admission (blue lines). Cerebral outcome was defined by cerebral performance category (CPC) as good; CPC 1–2 and poor; CPC 3–5. Data is shown as box plots with median as line and box indicating 25th–75th percentile and whiskers representing 10th–90th percentiles. Wilcoxon signed-rank paired test and non-paired Mann–Whitney *U* test. sTM; sThrombomodulin.

Syndecan-1 was the only endothelial marker that peaked on the day of admission, which is in-line with previous findings describing shedding of glycocalyx as an initial event in OHCA patients, where Syndecan-1 is an integral part.[27] While sSyndecan-1 correlated significantly with complement activation, it was not associated with outcome. Thus, our findings confirm results of a previous study in 163 comatose patients after OHCA, where sSyndecan-1 was not associated with mortality,[22] which may imply that sSyndecan-1 is an endothelial damage marker, but not necessarily associated with prolonged inflammatory reperfusion injury.

Complement system and endothelial cell activation have been shown to correlate with systemic cytokine release and haemodynamic status early post-cardiac arrest. The magnitude of this response has been associated with the severity of this post-cardiac arrest syndrome,[5,8] independent on TTM.[28] Likewise, endothelial activation and damage markers have been shown to be independent of TTM, except for sE-Selectin, which is lower in TTM36 treated patients.[22] The present study confirms this previously observed pattern of complement and endothelial activation and that endothelial activation reflected by thrombomodulin is associated with outcome,[22] although individual correlations of single parameters are relatively weak. In a study on 163 patients with one-time blood sampling within approximately two hours after OHCA, Cox proportional-hazard analyses found sE-selectin to be a univariate predictor of mortality.[29] Our findings add that sE-selectin is associated with outcome, also when assessed three days after OHCA. In addition, we show that sE-selectin is correlated with sCD14 at day three after cardiac arrest and not with the complement system, highlighting the multifactorial pathogenesis of the post-cardiac arrest syndrome. Unfortunately, due to large inter-patient variations seen in our and the aforementioned studies, thrombomodulin and sE-selectin are not reliable prognostic markers. Especially sE-selectin levels are affected by body-mass index.[30] However, both sE-selectin and thrombomodulin could be used in future clinical intervention studies assessing effect of an intervention by reduction of these endothelial activation markers.

Dual inhibition of both the complement system and CD14 in post-OHCA patients may be a general approach to attenuate the innate immune system broadly, preventing downstream overreaction of the

immune system including activation of endothelial cells, cytokine storm, and adaptive immune system,[31,32] and ought to be investigated further.

Limitations

This was a post-hoc analysis of a single-center study, which limits generalizability. Despite a rigorous sampling routine, blood was not acquired from 40 patients at day three, decreasing power of especially endothelial markers, which are known to be time-sensitive and increase later than complement activation markers. All patients were treated with the same TTM33 protocol reducing generalization to internationally recommended post resuscitation care, which includes TTM36. However, by focusing on TTM33, confounders based on quality of care were minimized. Time-to-ROSC is a pure quantitative parameter, and the quality of CPR, which is a known predictor of outcome, was not assessed.[33] This could therefore impact on the data. As this is an observational study, no cause-effect conclusions can be made, the study should thus be regarded as hypothesis generating, and may facilitate design of future studies investigating interventions in the immediate post-cardiac arrest phase.

Conclusion

In comatose, resuscitated OHCA patients, activation of the complement system is present in the majority of patients at admission. In particular, increased sC5b-9 was associated with poor outcome. The whole-body inflammation included subsequent endothelial cell activation and sCD14 release three days after OHCA.

Authorship contribution statement

Authors have contributed as follows to (1) Conception and design of the study (all authors), or acquisition of data (Nakstad, Stær-Jensen, Seljeflot, Lundqvist, Sunde, Andersen), or analysis and interpretation of data (Chaban, Schjalm, Vaage, Benth, Mollnes, Pischke), (2) drafting the article or revising it critically for important intellectual content (all authors), (3) final approval of the version to be submitted (all authors).

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Data statement

All anonymised data is available upon request from the corresponding author.

Conflict of interests

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.resuscitation.2021.05.038.

REFERENCES

1. Gräsner JT, Wnent J, Herlitz J, et al. Survival after out-of-hospital cardiac arrest in Europe — results of the EuReCa TWO study. Resuscitation 2020;148:218–26.
2. Nolan JP, Neumar RW, Adrie C, et al. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication. A Scientific Statement from the International Liaison Committee on Resuscitation; the American Heart Association Emergency Cardiovascular Care Committee; the Council on Cardiovascular Surgery and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the Council on Clinical Cardiology; the Council on Stroke. Resuscitation 2008;79:350–79.
3. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol 2012;229–317 Elsevier.
4. Laver S, Farrow C, Turner D, Nolan J. Mode of death after admission to an intensive care unit following cardiac arrest. Intensive Care Med 2004;30:2126–8.
5. Bro-Jeppesen J, Johansson PI, Kjaergaard J, et al. Level of systemic inflammation and endothelial injury is associated with cardiovascular dysfunction and vasopressor support in post-cardiac arrest patients. Resuscitation 2017;121:179–86.
6. Sekhon MS, Ainslie PN, Griesdale DE. Clinical pathophysiology of hypoxic ischemic brain injury after cardiac arrest: a "two-hit" model. Crit Care 2017;21:90.
7. Mentzelopoulos SD, Zakynthinos SG. Post-cardiac arrest syndrome: pathological processes, biomarkers and vasopressor support, and potential therapeutic targets. Resuscitation 2017;121:A12-a4.
8. Bottiger BW, Motsch J, Braun V, Martin E, Kirschfink M. Marked activation of complement and leukocytes and an increase in the concentrations of soluble endothelial adhesion molecules during cardiopulmonary resuscitation and early reperfusion after cardiac arrest in humans. Crit Care Med 2002;30:2473–80.
9. Nakstad ER, Staer-Jensen H, Wimmer H, et al. Late awakening, prognostic factors and long-term outcome in out-of-hospital cardiac arrest — results of the prospective Norwegian Cardio-Respiratory Arrest Study (NORCAST). Resuscitation 2020;149:170–9.
10. Bergseth G, Ludviksen JK, Kirschfink M, Giclas PC, Nilsson B, Mollnes TE. An international serum standard for application in assays to detect human complement activation products. Mol Immunol 2013;56:232–9.
11. Garred P, Mollnes TE, Lea T. Quantification in enzyme-linked immunosorbent assay of a C3 neoepitope expressed on activated human complement factor C3. Scand J Immunol 1988;27:329–35.
12. Mollnes TE, Lea T, Harboe M, Tschopp J. Monoclonal antibodies recognizing a neoantigen of poly(C9) detect the human terminal complement complex in tissue and plasma. Scand J Immunol 1985;22:183–95.
13. Perkins GD, Jacobs IG, Nadkarni VM, et al. Cardiac arrest and cardiopulmonary resuscitation outcome reports: update of the Utstein

Resuscitation Registry Templates for Out-of-Hospital Cardiac Arrest: a statement for healthcare professionals from a task force of the International Liaison Committee on Resuscitation (American Heart Association, European Resuscitation Council, Australian and New Zealand Council on Resuscitation, Heart and Stroke Foundation of Canada, InterAmerican Heart Foundation, Resuscitation Council of Southern Africa, Resuscitation Council of Asia); and the American Heart Association Emergency Cardiovascular Care Committee and the Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation. Circulation 2015;132:1286–300.
14. Böttiger BW, Motsch J, Braun V, Martin E, Kirschfink M. Marked activation of complement and leukocytes and an increase in the concentrations of soluble endothelial adhesion molecules during cardiopulmonary resuscitation and early reperfusion after cardiac arrest in humans. Crit Care Med 2002;30:2473–80.
15. Ricklin D, Reis ES, Lambris JD. Complement in disease: a defence system turning offensive. Nat Rev Nephrol 2016;12:383–401.
16. Haugaard SF, Jeppesen AN, Troldborg A, Kirkegaard H, Thiel S, AM Hvas. The complement lectin pathway after cardiac arrest. Scand J Immunol 2018;88:e12680.
17. Bisschops LL, van der Hoeven JG, Mollnes TE, Hoedemaekers CW. Seventy-two hours of mild hypothermia after cardiac arrest is associated with a lowered inflammatory response during rewarming in a prospective observational study. Crit Care 2014;18:546.
18. Bro-Jeppesen J, Jeppesen AN, Haugaard S, et al. The complement lectin pathway protein MAp19 and out-of-hospital cardiac arrest: Insights from two randomized clinical trials. Eur Heart J Acute Cardiovasc Care 2020;9:S145–52.
19. Orrem HL, Nilsson PH, Pischke SE, et al. Acute heart failure following myocardial infarction: complement activation correlates with the severity of heart failure in patients developing cardiogenic shock. ESC Heart Fail 2018;5:292–301.
20. Pischke SE, Gustavsen A, Orrem HL, et al. Complement factor 5 blockade reduces porcine myocardial infarction size and improves immediate cardiac function. Basic Res Cardiol 2017;112:20.
21. Wu W, Chopra A, Ziegler C, McLeod SL, Lin S. Predictive value of hospital discharge neurological outcome scores for long-term neurological status following out-of-hospital cardiac arrest: a systematic review. Resuscitation 2020;151:139–44.
22. Bro-Jeppesen J, Johansson PI, Hassager C, et al. Endothelial activation/injury and associations with severity of post-cardiac arrest syndrome and mortality after out-of-hospital cardiac arrest. Resuscitation 2016;107:71–9.
23. Arroyo-Espliguero R, Avanzas P, Jeffery S, Kaski JC. CD14 and toll-like receptor 4: a link between infection and acute coronary events? Heart (British Cardiac Society) 2004;90:983–8.
24. Marcos V, Latzin P, Hector A, et al. Expression, regulation and clinical significance of soluble and membrane CD14 receptors in pediatric inflammatory lung diseases. Respir Res 2010;11:32.
25. Yang Q, He G-W, Underwood MJ, Yu C-M. Cellular and molecular mechanisms of endothelial ischemia/reperfusion injury: perspectives and implications for postischemic myocardial protection. Am J Transl Res 2016;8:765.
26. Karpman D, Ståhl A-l, Arvidsson I, et al. Complement interactions with blood cells, endothelial cells and microvesicles in thrombotic and inflammatory conditions. Immune Responses to Biosurfaces 2015;19–42 Springer.
27. Grundmann S, Fink K, Rabadzhieva L, et al. Perturbation of the endothelial glycocalyx in post cardiac arrest syndrome. Resuscitation 2012;83:715–20.
28. Bro-Jeppesen J, Kjaergaard J, Wanscher M, et al. The inflammatory response after out-of-hospital cardiac arrest is not modified by targeted temperature management at 33 °C or 36 °C. Resuscitation 2014;85:1480–7.
29. Johansson PI, Bro-Jeppesen J, Kjaergaard J, Wanscher M, Hassager C, Ostrowski SR. Sympathoadrenal activation and endothelial damage are inter correlated and predict increased mortality in patients resuscitated after out-of-hospital cardiac arrest. A post Hoc sub-study of patients from the TTM-trial. PLoS One 2015;10:e0120914.
30. Schumacher A, Seljeflot I, Sommervoll L, Christensen B, Otterstad JE, Arnesen H. Increased levels of markers of vascular inflammation in patients with coronary heart disease. Scand J Clin Lab Invest 2002;62:59–68.
31. Barratt-Due A, Pischke SE, Nilsson PH, Espevik T, Mollnes TE. Dual inhibition of complement and Toll-like receptors as a novel approach to treat inflammatory diseases-C3 or C5 emerge together with CD14 as promising targets. J Leukoc Biol 2017;101:193–204.
32. Barratt-Due A, Pischke SE, Brekke OL, et al. Bride and groom in systemic inflammation–the bells ring for complement and Toll in cooperation. Immunobiology 2012;217:1047–56.
33. Nolan JP, Berg RA, Callaway CW, et al. The present and future of cardiac arrest care: international experts reach out to caregivers and healthcare authorities. Intensive Care Med 2018;44:823–32.