Complement Activation and its Prognostic role in Post-cardiac Arrest Patients

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Abstract

Cardiac arrest causes generalized ischaemia/hypoxia, and subsequent resuscitation inflicts reperfusion injury, the pathology of which is not fully understood. Moreover, predicting the prognosis of comatose, post-cardiac arrest patients is a complex clinical challenge. We hypothesized that the extent of complement activation might be a reliable predictor of mortality in this population. Forty-six comatose cardiac arrest patients were enrolled into our prospective cohort study, conducted in a tertiary care university clinic. All subjects were cooled to 32–34 °C body temperature for 24 h and then allowed to rewarm to normothermia. All patients underwent diagnostic coronary angiography. On admission, at 6 and 24 h, blood samples were taken from the arterial catheter. In these, complement products (C3a, C3, C4d, C4, SC5b9 and Bb) were measured by ELISA in blood samples. Patients were followed up for 30 days; 22 patients (47.8%) died by the end of this period. We observed that complement activation (determined as the C3a to C3 ratio) was higher in non-survivors than in survivors at each time point. In the multivariate Cox regression analysis, the C3a/C3 ratio determined 24 h after the initiation of therapeutic hypothermia predicted 30-day mortality regardless of age, sex and the APACHE II score. Complement activation occurs in post-cardiac arrest patients, and its extent correlates with 30-day survival. The C3a/C3 ratio might prove useful for estimating the prognosis of comatose post-cardiac arrest patients.

Introduction

Cardiac arrest causes generalized ischaemia/hypoxia, and subsequent resuscitation inflicts reperfusion injury. Although all the organs are damaged by hypoxia and hypoperfusion, cerebral injury occurs first. This is because the ischaemia tolerance and metabolic reserve of the brain are limited, and hence, its functions are greatly dependent on blood flow. Estimating the severity of brain damage and predicting the prognosis of comatose, post-cardiac arrest patients is a complex clinical challenge [1]. Nevertheless, several promising methods are available to foretell neurological outcome, preferably using a multimodal prediction approach [2]; however, further prognostic markers are needed [3].

Complement is an important component of the humoral immune system. It represents a highly effective means for the destruction of invading micro-organisms, for the elimination of immune complexes, as well as for the clearance of damaged host cells. Complement is activated by three pathways: the antibody-dependent classical pathway, the alternative pathway or the recently discovered ficolin-/mannose-binding lectin pathway [4]. Complement activation leads to the formation of opsonins (C3b), anaphylatoxins (C3a and C5a) and the cytolytic membrane attack complex (C5b9) [5]. A large body of evidence from the literature shows that complement activation plays an important role in the pathophysiology of cell and tissue damage after ischaemia and reperfusion [6]. The first report on the activation of complement after cardiac arrest appeared in 2002 [7]. Recently, Bisschops et al. [8] confirmed this observation during mild therapeutic hypothermia and rewarming. Several human ischaemic stroke studies also reported complement activation [9–11]. Moreover, the genetic deficiency of mannose-binding lectin (MBL) was associated with a better outcome after acute stroke in mice and humans [12]. This indicates that complement lectin pathway may play a crucial role in acute brain ischaemia.

Our group demonstrated previously that complement activation is associated with an unfavourable outcome after acute ischaemic stroke [13] and chronic heart failure (CHF)

[14]. Low ficolin-3 level is a major initiator of the ficolin–lectin pathway in complement activation [15]. We also showed a correlation between low ficolin-3 level, the severity and the unfavourable outcome of acute ischaemic stroke [16] and CHF [17]. We further presumed that as an integrative marker of cellular damage, complement activation might be an independent predictor of mortality in post-cardiac arrest patients. Accordingly, our study aimed to describe the changes of complement proteins and activation markers in post-cardiac arrest patients undergoing mild therapeutic hypothermia and to analyse its association with overall survival.

Materials and methods

Patient population, management and blood sampling. We performed a prospective cohort study of 46 consecutive comatose patients resuscitated after an out-of-hospital or an in-hospital cardiac arrest. The patients were managed at the Heart and Vascular Center, Semmelweis University, Budapest. All patients aged 18 years or older were eligible for inclusion, if they were comatose (Glasgow Coma Scale score ≤6) after the restoration of spontaneous circulation. Pregnant women were excluded along with patients who received thrombolytic therapy or required at least two vasopressors for refractory cardiogenic shock.

Intervention. All patients underwent diagnostic coronary angiography and percutaneous coronary intervention (PCI), where indicated. They were cooled to 32–34 °C by the rapid infusion of 30 ml/kg body weight cold (4 °C) lactated Ringer's solution, followed by external cooling with a water-circulating blanket (Blanketroll III, Cincinnati Subzero Medical Division, Cincinnati, OH, USA). Their body temperature was maintained at 32–34 °C for 24 h, followed by re-warming at a rate of 0.25–0.33 °C/h to normothermia (defined as core temperature ≥37 °C). Survival data were recorded at discharge and at 30 days. On admission to the intensive care unit [before the initiation of hypothermia (i.e. at 0 h)], as well as 6 and 24 h later, blood samples were drawn from the arterial catheter. All patients received antithrombotic and anticoagulant therapy; contrast media were used only during angiography. Blood samples [native and ethylenediaminetetraacetic acid (EDTA)] were centrifuged within 2 h (for 15 min at 2000 *g* and 25 °C), and the samples were stored at −80 °C until analysis. Demographic, prehospital and baseline data were collected on admission, whereas clinical data were recorded continuously. The Acute Physiology and Chronic Health Evaluation II (APACHE II) severity score was calculated retrospectively, based on clinical data from the initial 24 h.

The study was carried out in compliance with the Helsinki Declaration. The study protocol was approved by the local institutional review board (TUKEB), and written informed consent was obtained from the closest relative.

Measurement of biomarkers. The complement activation products C3a, C4d, SC5b9 and Bb were measured using commercial enzyme-linked immunosorbent assays, according to the manufacturer's instructions (Quidel, San Diego, CA, USA). To quantify C4 level, radial immunodiffusion was performed using polyclonal rabbit anti-human C4c (Dako, Glostrup, Denmark) and a human serum protein calibrator (Dako) as described previously [18]. C3 and total protein were determined with a Roche Integra 800 (Basel, Switzerland) analyzer. C3, C4 and total protein were measured in serum, while C3a, C4d, SC5b9 and Bb were measured in EDTA plasma.

Correction for hemodilution. Total protein level in the serum in post-cardiac arrest patients stayed low during the initial 24 h, due to vigorous volume replacement upon the initiation of hypothermia (rapid infusion of 30 ml/kg body weight cold lactated Ringer's solution). The medians and interquartile ranges of total protein concentrations were 60.3 (53.6–63.5), 55.3 (49.7–60.9) and 54.5 (48.3–58.2) g/l, at 0, 6 and 24 h, respectively. Therefore, the changes in total protein levels were used to normalize all biomarker concentrations, to adjust for the influence of excessive volume expansion. However, neither the C3a/C3 nor the C4d/C4 ratio required such correction, because of the division.

Statistical analysis. Statistical analysis was performed using GRAPHPAD PRISM version 5.0 (GraphPad Software, La Jolla, CA, USA) and SPSS v13.0 (SPSS Inc., Chicago, IL, USA). The data in the table are presented as medians, with 25th and 75th percentiles and ranges, because of their non-normal distribution. Continuous variables were compared between the two groups by Mann–Whitney's *U*-test, whereas Fisher's exact test was applied to categorical variables. The original data sets showed non-normal distribution. Changes over time in the two subsets (of survivors and of non-survivors) were analysed with two-way repeated-measures ANOVA, and the Bonferroni *post hoc* test was used in the case of log10 normalized data sets. Normality was tested with QQ plot. Homogeneity of variances was tested on the distribution of residuals. The assumptions of ANOVA were not violated. Survival was plotted according to the Kaplan–Meier method, and differences in survival between the groups were compared with log-rank tests. Univariate Cox proportional hazard regressions were calculated to predict mortality. Thereafter, the variable was fitted to multivariate Cox regression models, to assess its effect on survival after adjustment for covariates. The proportional hazards assumption was confirmed by examining plots of Schoenfeld residuals.

The results of the Cox regression models are presented as hazard ratios standardized on the SD of the predictors, as well as the corresponding 95% CI, Wald χ^2 and *P*-values of the likelihood-ratio tests. Two-tailed *P*-values were calculated, and the significance level was set at $P < 0.05$.

Results

Baseline characteristics of the cohort

Forty-six comatose patients were enrolled after a cardiac arrest. Twenty-two of them died within the first 30 days (non-survivors), whereas 24 were alive after this period (survivors). The median age and interquartile age range in survivors and in non-survivors were 57 (47–66) and 66 (61–69) years ($P = 0.014$), respectively. The severity score (APACHE II) was significantly lower in survivors than in non-survivors $P = 0.011$, Mann–Whitney's U-test. Both groups (survivors and non-survivors) were dominated by males (83% and 82%, $P = 1.00$) and characterized by a high out-of-hospital cardiac arrest rate (71% and 86%, $P = 0.28$, Fisher exact test). In our study, all patients underwent diagnostic coronary angiography; 79.2% of the survivors and 81.8% among non-survivors ($P = 1.00$, Fisher exact test) received percutaneous coronary intervention. Acute myocardial infarction was the underlying cause of cardiac arrest in more than 85% of the subjects. The cooling method was effective in both groups: the core temperature of 34 °C was achieved in all patients of both groups. The time from collapse to the start of ALS/BLS (advanced/basic life support), the time from the collapse to ROSC (return of spontaneous circulation) and the total duration of CPR (cardiopulmonary resuscitation) were not different between the groups of survivors and of non-survivors ($P = 0.62$, $P = 0.25$, $P = 0.49$, respectively; Mann–Whitney's U-test). The body mass index was similar in both groups (median 27.8 and 25.9 kg/m^2, $P = 0.71$, Mann–Whitney's U-test).

Complement components and activation products in post-cardiac arrest patients

First, we compared the levels of complement components and activation products at three different time points: on admission (0 h), as well as 6 and 24 h later, between the groups (of survivors and non-survivors, stratified according to 30-day survival), using repeated-measures ANOVA (Table 1).

We observed a significant change in the levels of complement activation products. Bb, sC5b9 and C3a/C3 ratio (a measure of C3 activation) decreased significantly during the initial 24 h of therapeutic hypothermia (Table 1, column 'Time').

Furthermore, we observed a consistently significant difference in C3a/C3 ratio between the groups (of survivors and of non-survivors), which suggests an association with the outcome (Table 1, column 'Group'). At each time point, the C3a/C3 ratio was higher in non-survivors than in survivors. The most important difference was observed at 24 h after the initiation of therapeutic hypothermia. Accordingly, we used the 24-h data for further analysis

to assess the impact of the C3a/C3 ratio on survival. No similar associations were observed in the case of any other complement components or activation products (Table 1).

The relationship between complement activation and survival

Next, we analysed whether the activation of C3 is associated with survival. The 46 patients were stratified according to the median of C3a/C3 ratio (measured at 24 h), into low- or high-C3a/C3 ratio groups. Kaplan–Meier survival analysis was carried out to evaluate mortality over time in both groups (Fig. 1). In our cohort, higher C3a/C3 ratio was associated with increased mortality (log-rank test, $P = 0.0043$, Fig. 1) during the 30-day-long follow-up period. C3a/C3 ratio, age and the severity score were also associated with 30-day mortality. Therefore, we performed multivariate Cox proportional hazards regression analysis to test the potential influence of confounders on the predictive role of the C3a/C3 ratio. In the univariate model, C3a/C3 ratio (considered as a continuous, 1-SD standardized variable) significantly predicted 30-day mortality (hazard ratio 1.747, 95% CI 1.218–2.505 for 1-SD increase; Table 2). In the multivariable Cox model, the prediction of 30-day mortality by the C3a/C3 ratio was adjusted for age, sex and APACHE II severity score. This parameter was an independent, significant predictor of mortality in adjusted models (hazard ratio 1.692, 95% CI 1.105–2.590 for 1-SD increase; Table 2). In summary, the C3a/C3 ratio predicts 30-day mortality in post-cardiac arrest patients regardless of age, sex and APACHE II severity score.

Discussion

The biochemical markers measured in blood samples can serve as valid and useful predictors of clinical outcomes in comatose cardiac arrest patients after successful resuscitation [1]. Moreover, measuring such markers might prove even more straightforward in everyday clinical practice than neuroimaging or electrophysiological testing [1]. In this study, we analysed for the first time whether the extent of complement activation – as measured by the C3a/C3 ratio – is a reliable biomarker for predicting all-cause mortality in comatose, resuscitated patients early, after the first 24-h period.

We observed complement activation in post-cardiac arrest patients and tested whether its extent is associated with 30-day survival. In our study, complement C3a/C3 ratio – a marker of complement activation – predicted short-term mortality (over 30 days) regardless of age, sex and APACHE II severity score.

Complement activation was already observed earlier in post-cardiac arrest patients [7, 8]. First, Böttiger et al. [7] reported significantly increased C3a and SC5b9 levels soon after cardiac arrest. Next, Bisschops et al. showed remark-

Table 1 Levels of complement proteins and activation markers in post-cardiac arrest patients and the results of repeated-measures ANOVA. The patients were stratified according to 30-day survival.

Original data sets Parameters	Time (h)	All patients ($n = 46$)	Survivors ($n = 24$)	Non-survivors ($n = 22$)	*P*-values of repeated-measures ANOVA on the log10 normalized data set		
					Group	Time	Interaction
C3a (ng/ml)	0	124.8 (97.1–239.3)[a]	120.9 (97.6–189.2)	148.4 (90.1–274.3)	0.104[b]	0.001	0.993
	6	103.2 (80.3–156.4)	96.3 (80.7–112.6)	119.4 (73.1–162.2)			
	24	116.4 (79.4–176.3)	114.5 (80.4–122.9)	174.3 (76.3–196.3)			
C3 (mg/ml)	0	1.06 (0.88–1.21)	1.11 (1.00–1.22)	1.01 (0.80–1.20)	0.063	0.007	0.014
	6	1.08 (0.86–1.20)	1.11 (1.01–1.23)	0.94 (0.80–1.12)			
	24	1.15 (0.94–1.27)	1.16 (1.08–1.27)	1.02 (0.86–1.23)			
C3a/C3	0	125.5 (86.8–215.6)	115.6 (85.4–196.1)	143.9 (106.4–281.0)	0.014	0.002	0.785
	6	105.8 (74.0–157.8)	87.5 (72.6–124.2)	150.2 (91.2–173.7)			
	24	101.3 (77.4–172.5)	95.5 (73.2–116.7)	172.5 (82.3–207.2)			
C4d (μg/ml)	0	1.7 (1.3–2.4)	1.8 (1.4–2.7)	1.5 (1.3–2.4)	0.834	0.146	0.643
	6	2.0 (1.4–2.4)	2.1 (1.6–2.6)	1.9 (1.3–2.3)			
	24	1.8 (1.3–2.2)	1.8 (1.3–2.2)	2.0 (1.3–2.4)			
C4 (mg/ml)	0	0.40 (0.31–0.49)	0.38 (0.30–0.50)	0.42 (0.33–0.48)	0.843	0.421	0.013
	6	0.43 (0.28–0.48)	0.41 (0.28–0.46)	0.44 (0.27–0.49)			
	24	0.42 (0.31–0.48)	0.42 (0.34–0.50)	0.40 (0.28–0.43)			
C4d/C4	0	4.4 (3.2–6.4)	5.6 (3.5–6.6)	4.1 (3.2–5.9)	0.938	0.084	0.204
	6	4.9 (3.8–6.7)	5.2 (3.7–7.9)	4.7 (4.0–5.9)			
	24	4.4 (3.2–5.8)	4.1 (3.2–4.6)	4.8 (3.9–6.3)			
Bb (μg/ml)	0	4.0 (2.1–8.5)	3.5 (2.1–7.0)	4.8 (2.3–9.4)	0.174	<0.001	0.676
	6	2.1 (1.4–3.8)	1.8 (1.4–2.8)	2.7 (1.2–4.0)			
	24	1.2 (0.8–2.0)	1.2 (0.8–1.7)	1.9 (1.0–2.3)			
sC5b9 (ng/ml)	0	342.9 (243.8–476.9)	363.6 (200.5–468.4)	312.3 (251.8–503.6)	0.582	<0.001	0.722
	6	193.6 (142.8–269.5)	185.3 (151.1–260.3)	193.8 (141.3–269.5)			
	24	225.1 (156.4–285.4)	225.1 (162.3–285.4)	225.3 (153.8–263.1)			

[a]Median values and interquartile ranges.
[b]*P*-values of repeated-measures ANOVA are presented.
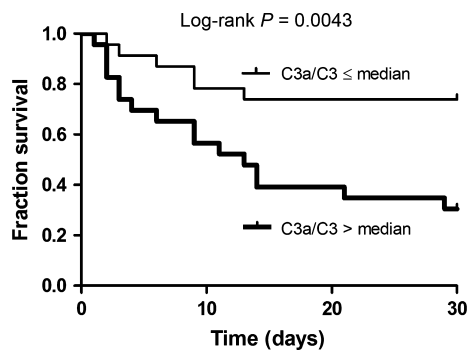
Figure 1 Kaplan–Meier survival plots in the two groups stratified according to the median of the C3a/C3 ratio, measured 24 h after admission. The *P*-value of log-rank test is indicated.

Table 2 Thirty-day mortality in post-cardiac arrest patients, as predicted by the increased C3a/C3 ratio determined 24 h after the initiation of therapeutic hypothermia.

Models	HR[a]	95% CI	χ^2	*P*-value
Univariate model of C3a/C3 ratio	1.747	1.218–2.505	7.504	0.006
Multivariate model of C3a/C3 ratio adjusted for age, sex and APACHE II score	1.692	1.105–2.590	5.249	0.022

[a]HR = Hazard ratio; HRs for variables shown as standardized hazard ratios (HR per 1 SD increase of the variable where SD = 87.02) with 95% CI; Wald χ^2 and *P*-values of likelihood-ratio tests are presented). C3a and C3 levels used in the C3a/C3 ratio were measured 24 h after the initiation of therapeutic hypothermia.

able complement activation in post-cardiac arrest patients. In particular, C3a, Bb and SC5b9 levels, which were increased on admission, decreased gradually during the mild hypothermia and increased again after rewarming [8]. In line with these observations, we found in the current study major complement activation: the C3a/C3 ratio, as well as Bb and sC5b9 levels decreased significantly both in survivors and in non-survivors. The C3a/C3 ratio was associated with the outcome, as it was markedly different

between the two groups. C3a activation has an important role in overall complement activation. Accordingly, our observation suggests that complement activation differs between survivors and non-survivors, it is rather important during the initial 24 h and it has a prognostic role. Furthermore, we observed declining levels of complement activation products (Bb and SC5b9); this might have been linked to core temperature during therapeutic

hypothermia. As Bb (alternative pathway) and sC5b9 (terminal pathway) are only parts of the activation cascade (in contrast to C3a, which has a central role in activation) unsurprisingly, their levels were not associated with the outcome.

Tissue necrosis or apoptotic cell death may be linked to the observed complement activation. However, alternative processes – such as those caused by severe hypoxia and ischaemia, followed by reperfusion – may also contribute to these reparatory and inflammatory processes. Remarkably, Bisschops et al. considered ischaemic cardiomyopathy the underlying cause of ventricular fibrillation in all of their patients, none of which required coronary angiography or percutaneous coronary intervention for myocardial infarction. On the contrary, all our patients underwent diagnostic coronary angiography, and most of them required percutaneous coronary intervention. Except for this difference, the major findings were rather similar between these studies, namely, complement activation was present, suggesting that it occurs regardless of the aetiology of cardiac arrest.

We found that the C3a/C3 ratio was associated with survival. Furthermore, we noticed a statistically significant trend in the decrease of the C3a/C3 ratio both in survivors and in non-survivors. This could have been linked to the decrease in core temperature during therapeutic hypothermia (Table 1).

In view of the associations between the covariates (age, sex, APACHE II severity score and C3a/C3 ratio) and mortality, we performed Cox regression analysis to investigate the potential influence of the former. We established that the C3a/C3 ratio determined at 24 h is an independent prognostic marker in post-cardiac arrest patients. When added to the baseline predictive model (age, sex and APACHE II severity score), the C3a/C3 ratio achieved a significant and independent improvement of the predictive model. This may indicate that the C3a/C3 ratio is good additive marker of cell damage and injury to the whole organism. Given the design of our study, it is impossible to locate the source of complement activation. We could not determine whether the C3a/C3 ratio is simply a surrogate marker for the total duration of ischaemia or else it reflects injury to specific organ system (s).

A large body of evidence from the literature shows the importance of complement activation in brain ischaemia, both after ischaemic stroke [9–13, 16] and following a cardiac arrest [7, 8]. Together, these studies confirm that complement activation is detectable in various forms of cerebral ischaemia. This marker could gain wide acceptance as a prognostic index or a determinant of risk status in the conditions mentioned herein.

Furthermore, amplification of the alternative pathway appears to be another important factor in complement activation in post-cardiac arrest patients. In this study, we have shown that the C3a/C3 ratio, determined in blood samples drawn soon after hospitalization, is suitable for the prediction of prognosis also in a post-cardiac arrest population.

Our study has its limitations, including the small number of study subjects. However, the statistical power of group estimations (Kaplan–Meier analysis) was sufficiently high in the case of the C3a/C3 ratio and mortality (Power = 0.79, at α = 0.05). Further, this was a single-centre study, and therefore, its results should be considered preliminary, until confirmed by independent studies.

Conclusions

Complement activation occurs in post-cardiac arrest patients, and the C3a/C3 ratio is associated with 30-day survival. The C3a/C3 ratio determined at 24 h predicted 30-day mortality regardless of age, sex and APACHE II score. Therefore, it could prove useful for estimating the prognosis of comatose post-cardiac arrest patients.

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Conflict of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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