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ORIGINAL RESEARCH ARTICLE

Acute heart failure following myocardial infarction: complement activation correlates with the severity of heart failure in patients developing cardiogenic shock

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Abstract

Aims Heart failure (HF) is an impending complication to myocardial infarction. We hypothesized that the degree of complement activation reflects severity of HF following acute myocardial infarction.

Methods and results The LEAF trial (LEvosimendan in Acute heart Failure following myocardial infarction) evaluating 61 patients developing HF within 48 h after percutaneous coronary intervention-treated ST-elevation myocardial infarction herein underwent a *post hoc* analysis. Blood samples were drawn from inclusion to Day 5 and at 42 day follow-up, and biomarkers were measured with enzyme immunoassays. Regional myocardial contractility was measured by echocardiography as wall motion score index (WMSI). The cardiogenic shock group (n = 9) was compared with the non-shock group (n = 52). Controls (n = 44) were age-matched and sex-matched healthy individuals. C4bc, C3bc, C3bBbP, and sC5b-9 were elevated in patients at inclusion compared with controls ($P < 0.01$). The shock group had higher levels compared with the non-shock group for all activation products except C3bBbP ($P < 0.05$). At Day 42, all products were higher in the shock group ($P < 0.05$). In the shock group, sC5b-9 correlated significantly with WMSI at baseline (r = 0.68; P = 0.045) and at Day 42 (r = 0.84; P = 0.036). Peak sC5b-9 level correlated strongly with WMSI at Day 42 (r = 0.98; P = 0.005). Circulating endothelial cell activation markers sICAM-1 and sVCAM-1 were higher in the shock group during the acute phase ($P < 0.01$), and their peak levels correlated with sC5b-9 peak level in the whole HF population (r = 0.32; P = 0.014 and r = 0.30; P = 0.022, respectively).

Conclusions Complement activation discriminated cardiogenic shock from non-shock in acute ST-elevation myocardial infarction complicated by HF and correlated with regional contractility and endothelial cell activation, suggesting a pathogenic role of complement in this condition.

Keywords Complement activation; Inflammation; Myocardial infarction; Acute heart failure; Cardiogenic shock; Wall motion score index

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Introduction

The current therapeutic strategy with rapid restoration of blood flow to the ischaemic myocardium by percutaneous coronary intervention (PCI) has markedly reduced the short-term and long-term morbidity and mortality in acute ST-elevation myocardial infarction (STEMI).[1,2] However, acute heart failure (HF) and cardiogenic shock are still important clinical complications of STEMI and remains the leading cause of death in patients with acute myocardial infarction (MI).[3–5] Cardiogenic shock is

defined as a state of mismatch between oxygen delivery and oxygen demand caused by critical tissue hypoperfusion due to reduced cardiac output, and the diagnosis is based on haemodynamic (e.g. hypotension), clinical (e.g. cold extremities), and biochemical (e.g. increased lactate) criteria.[6]

Acute coronary syndromes and MI are associated with inflammation,[7] and activation of the innate immune system such as Toll-like receptors and the complement system are implicated in mediating both adaptive (e.g. tissue repair) and maladaptive (e.g. cardiomyocyte necrosis and apoptosis) responses.[8–10] Cardiogenic shock following MI would exaggerate the inflammatory responses by tissue hypoperfusion and potentially induce a vicious circle.[11] Current management of cardiogenic shock involves strategies to increase cardiac output and antithrombotic treatment but do not target the inflammatory response *per se*.[3]

The complement system, for long appreciated only as a first line of defence against microbes, is today acclaimed for immune surveillance by much broader means. Damage-associated molecular patterns can trigger complement activation through three characterized pathways: the classical, the lectin, and the alternative pathway. They all merge at the central complement component C3 and continue into a common terminal pathway with cleavage of C5 and formation of the terminal C5b-9 complement complex, which, when inserted into membranes as the membrane attack complex, can lyse bacteria and activate host cells. The soluble form of C5b-9 (sC5b-9) is a fluid-phase marker indicating that the terminal pathway has been activated to its very end.[12]

Whereas a balanced activation of the complement system is regarded as beneficial for the host, an overwhelming activation could promote sustained inflammation and tissue damage, as seen during MI and the following ischaemia/reperfusion injury,[13] but its relation to acute HF development following MI is not clear. However, complement is activated in patients with chronic HF, regardless of aetiology, potentially associated with unfavourable outcome,[14–16] and recent studies have highlighted the activation of the lectin pathway as central in ischaemic heart disease and chronic HF.[17,18]

The present study is a *post hoc* study of the LEAF (LEvosimendan in Acute heart Failure following myocardial infarction) trial,[19] an interventional study on patients developing HF within 48 h following PCI-treated STEMI. We hypothesized that enhanced complement activation could be a hallmark of acute HF in this patient group and may discriminate between HF with or without cardiogenic shock.

Materials and methods

Study design and population

The patient population and study design in the LEAF trial have previously been described in detail.[19] Briefly, 61 patients with PCI-treated STEMI who (i) had successful opening of the occluded coronary artery, (ii) had decreased wall motion in at least 3 of 16 segments of the left ventricle evaluated by echocardiography, and (iii) developed clinical signs of HF within 48 h (range: 14–33 h) following PCI were randomized to treatment with the calcium sensitizer levosimendan or placebo.[19] HF was defined as dyspnoea at rest and the presence of at least one of the following symptoms: pulmonary oedema, signs of pulmonary congestion on X-ray, need for continuous positive airway pressure or mechanical ventilation, or need for intravenous diuretics due to symptoms of congestion or persistent oliguria (urine output $<$0.5 mL/kg/h) after volume therapy. Criteria for subgrouping patients into cardiogenic shock included both of the following: (i) systolic blood pressure $<$ 90 mmHg after 60 min of volume therapy or systolic blood pressure 90–100 mmHg despite vasoactive support and (ii) signs of organ hypoperfusion such as cold and clammy extremities, oliguria, or reduced consciousness. Exclusion criteria were septic shock, acute respiratory distress syndrome, creatinine $>$ 450 μmol/L, severe hepatic failure, age $<$ 20 years, heart rate $>$ 120 b.p.m., pregnancy, significant mechanical outflow obstruction, haemoglobin $<$ 8 g/dL, or allergy to the study medication or any of its components.

In the present study, the STEMI patients who developed cardiogenic shock (n = 9) were compared with patients with HF without any signs of cardiogenic shock (n = 52) in order to investigate differences in complement activation between severe and less severe degree of HF. For comparison, blood samples were obtained from 44 age-matched and sex-matched healthy controls. Importantly, to ensure that treatment with levosimendan did not affect the degree of complement activation, we compared the two treatment groups with respect to sC5b-9 over the whole study period. There was no significant difference between the groups (P = 0.72), and they were thereafter handled as one population.

Blood sampling protocol

Blood samples were collected from patients at the time of inclusion (Day 0), that is at time of HF diagnosis (median 24 h following PCI) and at Days 1, 2, 5 (acute phase of the disease), and 42 following inclusion (follow-up sample) as previously described.[19] Briefly, blood samples were collected in ethylendiaminetetraacetic acid (EDTA), citrate, and serum vacutainer tubes (BD, Plymouth, UK). EDTA and citrated plasma samples were stored on crushed ice immediately after sampling and centrifuged within 30 min at 3000 *g* for 20 min at 4°C to obtained platelet-poor plasma. Blood for serum preparation was allowed to clot for 60 min in room temperature and thereafter centrifuged at 2500 *g* for 10 min for

isolation of serum. All samples were stored at −80°C until analysed and thawed only once.

Assays for complement activation markers

The complement activation products C4bc (classical and lectin pathway), C3bc (common pathway), C3bBbP (alternative pathway), and sC5b-9 (terminal pathway) were measured in EDTA-plasma samples from patients and controls by in-house enzyme-linked immunosorbent assays. All assays are based on either monoclonal antibodies detecting activation-specific neoepitopes (C4bc, C3bc, and C5b-9) or pairs of antibodies detecting complexes formed between single components upon activation (C3bBbP) as previously described in detail.[20] The level of the respective marker was related to the International Complement Standard #2, defined to contain 1000 complement arbitrary units per millilitre.[20]

Lectin pathway recognition molecules

Plasma concentrations of mannose-binding lectin (MBL), ficolin-1 (FCN1), ficolin-2 (FCN2), and ficolin-3 (FCN3) were determined by sandwich enzyme-linked immunosorbent assays using specific in-house produced monoclonal antibodies as previously described.[21–24]

Markers of endothelial activation

Levels of soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) of the current material have previously been analysed in serum and published.[25] In the present study, we extended the data analyses by comparing these markers between patients with and without cardiogenic shock, to explore whether they corresponded with the degree of HF and whether there were any correlations between these markers and markers of complement activation.

Echocardiography

Left ventricular function was measured as wall motion score index (WMSI) by echocardiography as previously described.[19] A 16-segment model was used where a normally contracting or hyperkinetic segment was given a score of 1, a hypokinetic segment scored 2, akinesia gave a score of 3, and a dyskinetic segment scored 4 points. WMSI was calculated by dividing the sum of scores by the number of segments scored. All examinations were performed by two experienced echocardiographers on Days 0, 1, and 42, and the analyses were performed by one observer. An ultrasonic device system (Vivid i or Vivid 7, GE Vingmed Ultrasound, Horten, Norway) was used for the examinations, and the analyses were performed with dedicated software (Echopac GE Vingmed Ultrasound).

Infectious complications

In order to test whether infectious complications contributed to activation of the complement system, levels of activation makers were compared in patients with documented or suspected infection, based on positive culture testing, X-rays, and clinical evaluation (n = 14), to patients without infection (n = 38). This comparison was only performed in the non-shock group because the cardiogenic shock group did not include enough patients to ensure statistical testing. Statistical tests for correlation between complement activation and biochemical markers of infection [C-reactive protein, white blood cell (WBC) count, or interleukin (IL)-6] were also performed.

Data presentation and statistics

In addition to the patient cohort, a control group comprising 44 age-matched and sex-matched healthy individuals was included. The patient cohort was divided into two groups: one group consisting of patients who developed HF without any signs of cardiogenic shock, the non-shock group (n = 52), and one group consisting of patients who developed cardiogenic shock, referred to as the shock group (n = 9).[19] Differences between these two groups during the first 5 days after inclusion (Days 0–5) were analysed with linear mixed model analyses. Differences between the two groups were tested with t-test or alternatively with the Mann–Whitney U-test when data were not normally distributed. To compare categorical data between groups, the χ^2 test or Fisher's exact test was used. Differences between more than two groups were tested with Kruskal–Wallis test using Dunn's *post hoc* test. Bonferroni correction was used to correct for multiple testing. Correlation analyses were measured by the Spearman correlation test. All results are given as mean and standard error of the mean. A P value of <0.05 was considered statistically significant. IBM SPSS Statistics version 21 (Armonk, NY) was used for analysis, while GraphPad Prism version 6 (San Diego, CA) was used for data presentation.

Ethics

The study was approved by The Regional Ethics Committee South-Eastern Norway Regional Health Authority, and the study was conducted in accordance with the principles of the Declaration of Helsinki (clinicaltrials.gov NCT00324766). All patients provided written informed consent. If a patient was unable to give informed consent, relatives were informed, and a written consent was acquired from the patient as soon as possible.

Results

Complement activation

Sixty-one patients were included in the study, and those who developed cardiogenic shock (n = 9) were compared with patients with HF without any signs of cardiogenic shock (n = 52) (*Table 1*). At the time of inclusion, C4bc, reflecting classical and lectin pathway activation, C3bc, reflecting C3 activation, C3bBbP, reflecting activation of the alternative pathway, and sC5b-9, reflecting the terminal pathway activation, were significantly elevated in the patient cohort (n = 61) compared with the healthy controls (n = 44) ($P < 0.05$ for all; *Figure 1A–D*). Patients developing shock had significantly higher levels of C4bc, C3bc, and sC5b-9 in the acute phase of the disease (Days 0–5), compared with patients without shock ($P < 0.05$ for all; *Figure 1A, B,* and *D*). Even at Day 42, there was an enhanced complement activation reflected by higher levels of all four activation markers (C4bc, C3bc, C3bBbP, and sC5b-9) in the shock group compared with the non-shock group ($P < 0.05$ for all; *Figure 1E–H*).

Lectin pathway recognition molecules

The level of FCN2 was at the time of inclusion lower among patients vs. controls ($P < 0.05$), whereas no significant differences were observed for MBL, FCN1, or FCN3 (*Figure 2A–D*). During the acute phase of the disease (Days 0–5), FCN2 increased significantly ($P < 0.05$) in the patient cohort as a whole, but there were no significant group differences between those with and without cardiogenic shock (*Figure 2*C). At Day 42, however, the shock group had a significantly higher level of FCN2 compared with the non-shock group ($P < 0.05$; *Figure 2G*). No significant group differences were found for MBL, FCN1, or FCN3 (*Figure 2E, F,* and *H*). Furthermore, there was no correlation between C4bc and MBL or the ficolins.

Markers of endothelial activation

We have previously published data on endothelial activation in these patients.[25] When now analysing their relation to cardiogenic shock, we found that sICAM-1 and sVCAM-1 were significantly higher in the shock group compared with the non-shock group during the acute phase of the disease (Days 0–5) ($P < 0.01$ for both; *Figure 3A,B*) with no significant differences at Day 42, (*Figure 3C,D*).

Correlation between complement activation and regional myocardial contractility

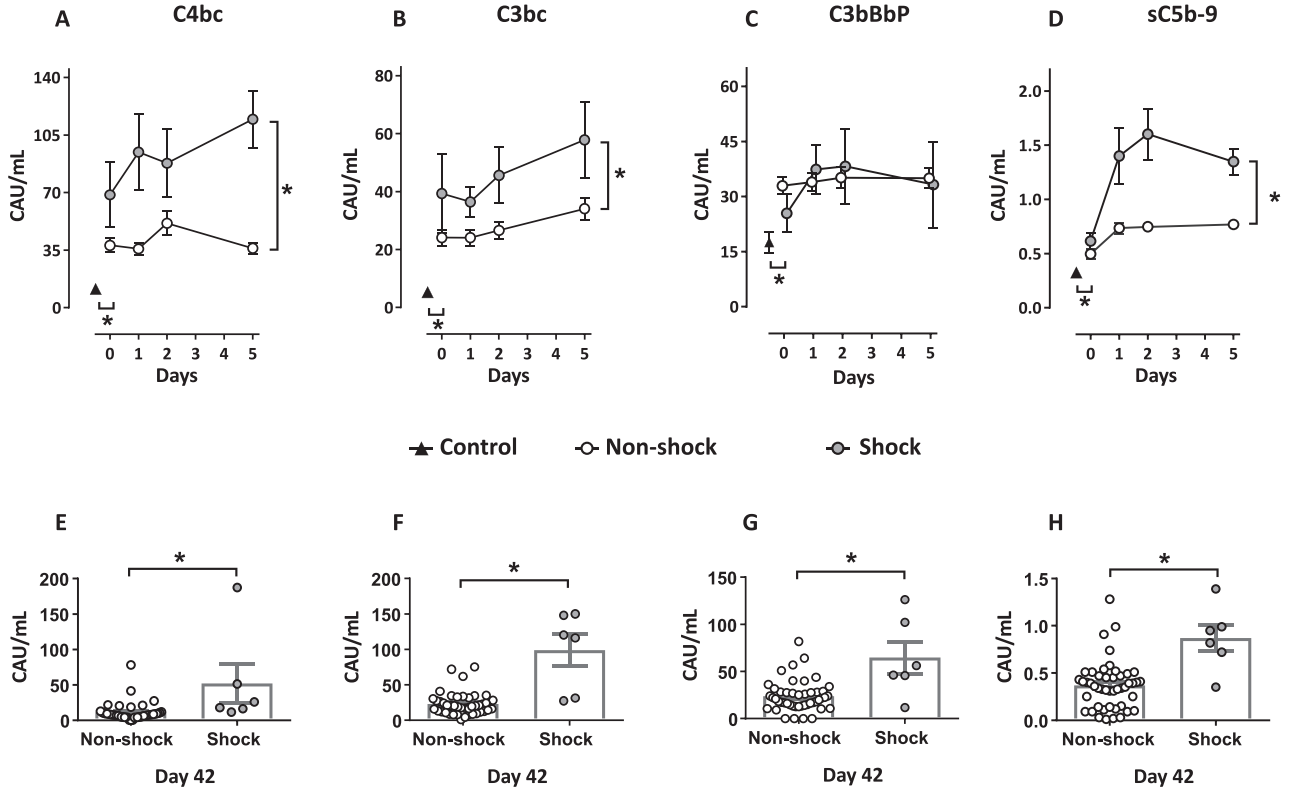
In the shock group, there was a significant correlation between complement activation as measured by sC5b-9 and

Table 1 Baseline characteristics of 61 patients with ST-elevation myocardial infarction developing acute heart failure with or without cardiogenic shock

	Shock	Non-shock	*P* value
Total number (female/male)	9 (3/6)	52 (15/37)	0.89
Age (years, mean, and range)	57 (49–68)	66 (56–74)	0.08
TnT[a] (ng/L)	14 640 (7580–20 925)	12 279 (7811–16 607)	0.43
Creatinine[a] (μmol/L)	81 (52–150)	82 (69–95)	0.91
eGFR[a] (mL/min/m^2)	60 (33–60)	60 (60–60)	0.24
NT-proBNP[a] (pmol/L)	315 (202–721)	463 (266–840)	0.52
C-reactive protein[a] (mg/L)	40 (24–100)	57 (35–97)	0.42
WBC count (×10^9/L)[a]	11 (8.9–17)	12 (10–15)	0.49
IL-6[a] (pg/mL)	29 (19–40)	27 (21–33)	0.54
Previous hypertension, *n* (%)	5 (56)	16 (31)	0.15
Previous dyslipidemia, *n* (%)	1 (11)	12 (23)	0.42
Current smoking, *n* (%)	6 (67)	16 (30)	<0.05
Previous diabetes mellitus, *n* (%)	1 (11)	5 (10)	0.89
Previous statin treatment, *n* (%)	2 (22)	13 (25)	0.86
Previous myocardial infarction, *n* (%)	3 (33)	8 (15)	0.20
Multi-vessel disease, *n* (%)	5 (56)	26 (44)	0.76
Atrial fibrillation[a], *n* (%)	1 (9)	1 (2)	0.16
Systolic blood pressure[a], mmHg	85 (72–94)	106 (96–117)	<0.001
Diastolic blood pressure[a], mmHg	55 (48–58)	67 (60–72)	<0.001
Hours from symptom start to PCI	3 (2–8)	3 (2–6)	0.80
Hours from PCI to baseline	17 (10–23)	23 (14–32)	0.07
LVEF[a], %	44 (34–49)	41 (38–47)	0.88
Antimicrobial treatment, *n* (%)	8 (89)	14 (27)	<0.001
Mortality within 6 months, *n* (%)	3 (33)	2 (4)	<0.05

GFR, glomerular filtration rate; IL-6, interleukin 6; LVEF, left ventricular ejection fraction; NT-proBNP, N terminal pro brain natriuretic peptide; PCI, percutaneous coronary intervention; TnT, troponin T; WBC, white blood cell.
Data are given as median (25th and 75th percentile) or number (%).
[a]At the time of inclusion, that is median 24 h following PCI.

Figure 1 Complement activation products during the first 5 days of the disease and at Day 42 after inclusion. Figures in the upper panel (A–D) show values at inclusion and throughout the acute phase of the disease (Days 0–5). (A) Plasma levels for C4bc (classical and lectin pathway activation), (B) C3bc (common activation of all initial pathways), (C) C3bBbP (alternative pathway activation), and (D) sC5b-9 (terminal pathway activation) are shown for patients with cardiogenic shock (n = 9, grey circles), patients with heart failure without cardiogenic shock (n = 52, open circles), and healthy controls (n = 44, black triangles). Statistical differences between the shock group and the non-shock group of patients from inclusion (Day 0) to Day 5 (the acute phase of the disease) are indicated with brackets and *($P < 0.05$) at the right-hand side of the graph. Statistical difference between patients and controls at the time of inclusion are indicated with *($P < 0.05$). Figures at the lower panel (E–H) show plasma levels at Day 42 for (E) C4bc, (F) C3bc, (G) C3bBbP, and (H) sC5b-9 for patients with cardiogenic shock (n = 7, grey columns) and patients without cardiogenic shock (n = 45, white columns). Data are given as mean ± standard error of the mean. CAU, complement arbitrary units.



WMSI at the day of inclusion (Day 0) (r = 0.678, P = 0.045) and at Day 42 (r = 0.841, P = 0.036; *Table 2*). At these two time points, both blood sampling and WMSI were performed, and thus, direct correlation tests could be performed. sC5b-9 reached its highest level at Day 2, where WMSI was not performed. Interestingly, this peak sC5b-9 level correlated significantly with WMSI at Day 42 in the shock group (r = 0.975, P = 0.005; *Table 2*). Weaker or no correlations were found for the other complement activation products: WMSI Day 1 correlated with C3bBbP measured at Days 2 (r = 0.943, P = 0.005) and 42 (r = 0.829, P = 0.042), and WMSI measured at Day 0 correlated with C4bc measured at Day 0 (r = 0.703, P = 0.035, data not shown). No correlations were found between WMSI and C3bc. In the non-shock group, the only significant correlation was found between C4bc measured at Day 1 and WMSI at Day 0 (data not shown).

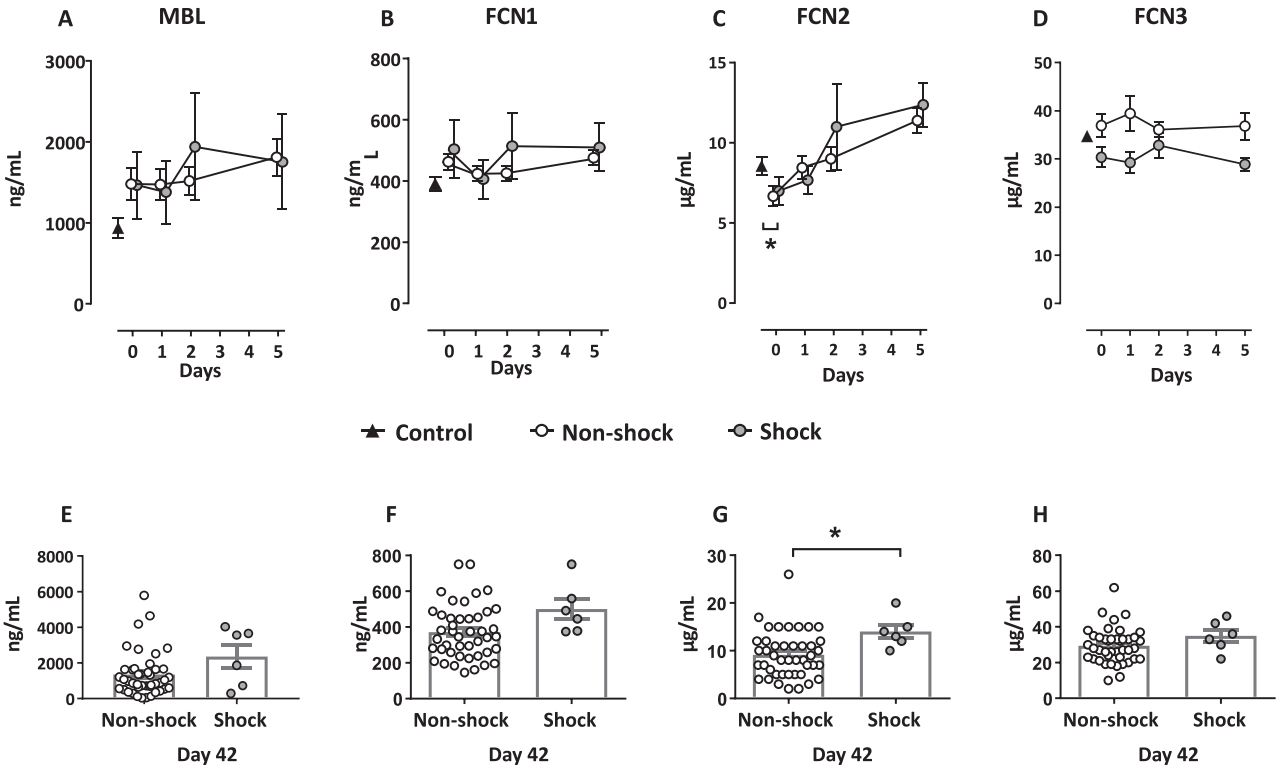
Correlation between complement activation and the markers of endothelial activation

There was a significant correlation between complement activation in the whole patient group (n = 61) at Day 2, when sC5b-9 peaked, and peak level of sVCAM-1 (r = 0.296, P = 0.022) and sICAM-1 (r = 0.317, P = 0.014), whereas no correlation was found when the shock and non-shock groups were analysed separately.

Complement activation following infection

There was no significant difference in complement activation, measured as sC5b-9, between patients with infection (n = 14) and patients without infection (n = 38) in the non-shock group during the acute phase of the disease (Days 0–5) (P = 0.44).

Figure 2 Lectin pathway proteins levels during the first 5 days of the disease and at Day 42. Figures at the upper panel (A–D) show plasma levels for the (A) mannose-binding lectin (MBL), (B) Ficolin-1 (FCN1), (C) Ficolin-2 (FCN2), and (D) Ficolin-3 (FCN3) for patients from inclusion (Day 0) to Day 5 (the acute phase of the disease). Figures in the lower panel (E–H) show plasma levels for the (E) MBL, (F) FCN1, (G) FCN2, and (H) FCN3 at Day 42. The figures are shown with the same patient populations and details as described in the *Figure 1* legend. $*P < 0.05$.



There was no difference between the non-shock and the shock group with respect to levels of C-reactive protein, WBC count, or IL-6 (*Table 1*). We found no association between peak values of sC5b-9 and markers of infection (C-reactive protein, WBC count, or IL-6, all measured as peak values) (*Table S1*).

Discussion

In the present study, we found increased complement activation in patients who developed acute HF following PCI-treated STEMI compared with healthy controls. Notably, the degree of complement activation discriminated those patients developing cardiogenic shock from those in the non-shock group. The increased activation persisted even 6 weeks after STEMI in the shock group. In these patients, there was also a strong correlation between complement activation and regional contractility measured as WMSI both at inclusion and at 6 weeks. Although complement activation has been shown to be involved in the progress of HF, this is, to the best of our knowledge, the first study to document that the degree of complement activation is directly related to the disease severity and impaired myocardial function in patients developing acute HF following STEMI.

The patient population in this study was characterized by large MIs determined by high levels of troponins and clinical and echocardiographic findings.[19] In the present study, we show that the complement activation products C4bc, C3bc, C3bBbP, and sC5b-9, representing complement activation from initiation to terminal activation, were increased at the time when the patients were diagnosed with HF (14–33 h following PCI treatment), compared with healthy individuals. Furthermore, there was stronger and more persistent complement activation in the most severely affected patients. This persistent activation indicates that complement might play an important role in the pathophysiological process of HF. In fact, the peak level of sC5b-9 during the acute phase correlated significantly with WMSI after 6 weeks, suggesting that complement-mediated mechanisms could promote myocardial damage with subsequent development of severe HF following STEMI.

Because of its amplification loop, the alternative pathway can contribute substantially to complement activation from the level of C3 and further downstream the activation cascade.[26,27] The lack of difference between the two patient groups with respect to the activation product C3bBbP is therefore somewhat surprising. The amplification loop is, however, under strict control by regulatory proteins like factor H, and complement activation triggered presuming via the lectin pathway with a tight

Figure 3 Serum levels of the endothelial cell activation markers sICAM-1 and sVCAM-1 during the first 5 days of the disease and at Day 42. Figures in the upper panel show serum levels of the (A) soluble intercellular adhesion molecule 1 (sICAM-1) and the (B) soluble vascular adhesion molecule 1 (sVCAM-1) for patients from inclusion (Day 0) to Day 5 (the acute phase of the disease). Figures in the lower panel show serum levels of (C) sICAM-1 and (D) sVCAM-1 at the control measurement at Day 42. The figures are shown with the same patient populations and details as described in the *Figure 1* legend. $^{*}P < 0.05$.

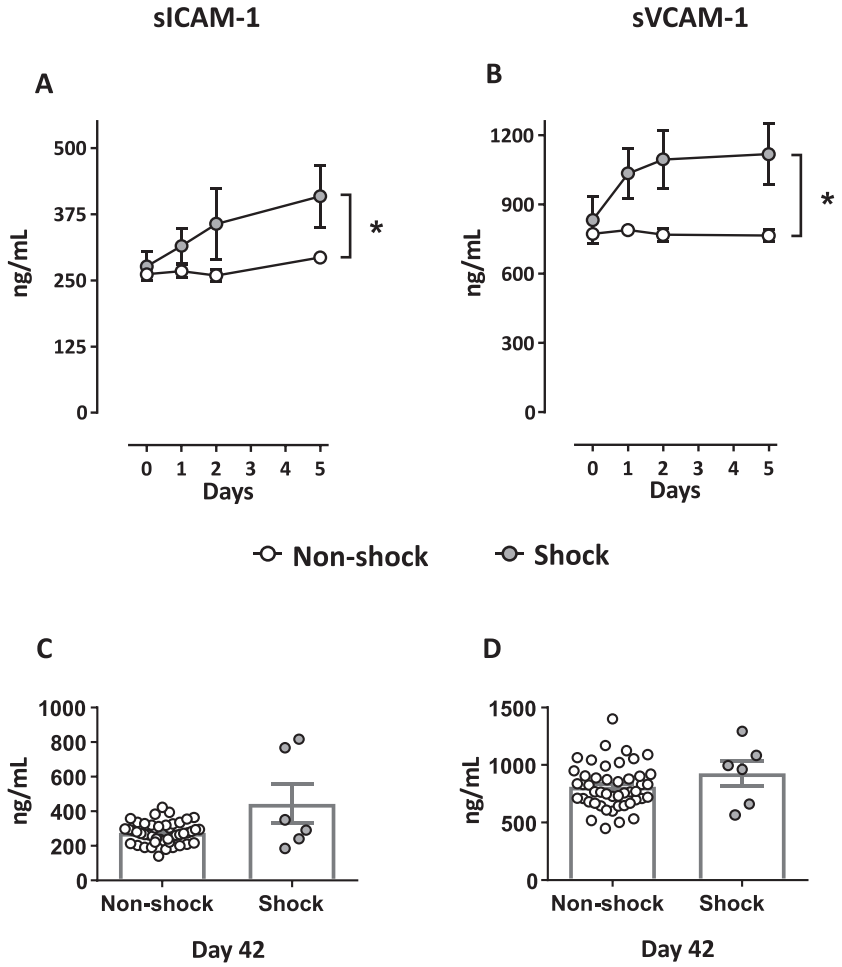


Table 2 Correlation between left ventricular regional contractility measured as wall motion score index and complement activation measured by sC5b-9 in patients with cardiogenic shock ($n = 9$) following percutaneous coronary intervention-treated ST-elevation myocardial infarction

		sC5b-9 Day 0	sC5b-9 Day 2	sC5b-9 Day 42
WMSI Day 0	*r*	0.678	0.206	0.522
	P	**0.045**	0.696	0.288
WMSI Day 1	*r*	0.311	0.812	0.488
	P	0.415	0.050	0.329
WMSI Day 42	*r*	0.551	0.975	0.841
	P	0.257	**0.005**	**0.036**

WMSI, wall motion score index. Statistical significance is shown in bold.

regulatory control of the alternative pathway in both groups in the early phase of disease may be a reasonable explanation for this finding. The regulatory balance may then have changed after the initial phase, explaining the significant difference in C3bBbP in the two groups at Day 42.

Several clinical and experimental studies have previously demonstrated increased complement activation in cardiovascular disease and HF.[14,15,28–32] Particularly, the lectin pathway has been linked to complement-mediated myocardial injury and HF,[18,33–35] and lectin pathway recognition molecules were therefore thoroughly investigated in the present study. MBL and the ficolins are circulating recognition molecules binding to molecular structures on damaged host cells further activating the mannose-binding serine proteases, MASP1 and MASP2.[36]

MBL is also an acute phase reactant,[37] and altered levels of FCN1–3 are reported in various pathological conditions, either due to consumption or changed expression.[38] The lower level of FCN2 in the patient population at inclusion compared with healthy controls is in line with a previous observation seen in STEMI patients[35] and is suggested to reflect consumption in the early phase of the disease. From the inclusion level, FCN2 increased significantly during the first 5 days of the disease and was at Day 42 significantly higher in the shock group. The other recognition molecules, MBL, FCN1, and FCN3, did not differ significantly from the healthy controls at inclusion. FCN1 was higher than the controls, although not significant, and showed no change during the course. The reason for the different patterns for FCN1 and FCN2 is uncertain but might be related to their different profiles for release and consumption, which makes it difficult to compare these two. FCN1 is synthesized by peripheral leukocytes. Upon cell activation, secretion of FCN1 increases, but the majority is tethered to the cell membrane of the activated cell.[39] This can explain the small, however non-significant, early increase of FCN1 in patients. FCN2 is synthesized in the liver as a soluble protein.[40] Increased secretion of FCN2 is, in relation to FCN1, delayed, which enables a consumption profile early after MI. Further on, FCN1 and FCN2 are highly homologous, but FCN2 has four carbohydrate-binding domains, whereas FCN1 has only one.[41] FCN2 might therefore bind its ligand more tightly as compared with FCN1, but without knowing the exact target, this remains speculative.

C4bc reflects both classical and lectin pathway activation. Although classical pathway activation cannot be excluded, our findings of increased C4bc is in accordance with lectin pathway activation during the acute phase,[33,42] although the role of the lectin pathway in post-MI HF is still elusive.

Microbial infections are well-known activators of the complement system.[43] We therefore compared complement activation in patients with or without signs of infection. The non-shock group contained a sufficient amount of patients treated for infections, documented or suspected, to enable statistical analysis regarding infectious complications and complement activation. Notably, there was no difference in complement activation in patients with or without infection in this group. Antibiotics were given mainly because of suspected aspiration, and septic patients were excluded from the trial. Furthermore, there were no correlations between peak levels of sC5b-9, C-reactive protein, IL-6, or WBC count. Thus, there is no evidence that the increased complement activation is caused by infections but rather by the cardiogenic shock *per se*.

The patients in the shock group were characterized by significantly increased levels of the soluble adhesion molecules sVCAM-1 and sICAM-1 as compared with the non-shock group reflecting enhanced endothelial cell activation in those with the most severe HF. Activated endothelial cells have been shown to secrete complement components and to express adhesion molecules ICAM-1 and VCAM-1 in response to sC5b-9 and are also targets for complement activation products.[44] Herein, we also found a significant correlation between sC5b-9 and the adhesion molecules in the whole HF group, further suggesting crosstalk between endothelial cells and terminal complement activation in patients with acute, severe HF following MI. With a positive correlation of sustained complement activation and development of cardiogenic shock, the critical question arises whether complement activation solely is the result of hypoperfusion caused by cardiogenic shock, or whether it also contributes to exacerbation of shock and, in extension, if these patients would benefit from complement inhibition. Increased systemic complement activation has previously been shown in patients with chronic HF consistent with tissue hypoperfusion, acidosis, and endothelial cell damage.[14] Neoantigens exposed in ischaemic tissue are linked to recognition by natural IgM and subsequent lectin pathway activation,[45] which would support sustained complement activation. If complement significantly aggravates the shock syndrome, there would be fear for a vicious circle. By being part of the innate immune system, complement is instantly activated upon 'danger' and has the potential for initiating a broad range of inflammatory responses. Specific complement inhibition may therefore be suitable in patients where attenuation of inflammation is desired, including patients with post-MI HF and particularly those with cardiogenic shock. Various clinical trials targeting different parts of the inflammatory response have failed to reach significance with regard to their primary endpoints.[46] However, in the COMplement inhibition in Myocardial infarction treated with Angioplasty trial,[47] where complement inhibition with the C5-inhibitor pexelizumab was given as a bolus dose and with continuous infusion for 20 h following MI, a significant reduction in 90 day mortality was seen. The incidence of cardiogenic shock was reduced with 45%, however, non-significantly. The Assessment of Pexelizumab in Acute Myocardial Infarction trial did not show any effect of pexelizumab,[48] but there is a remaining question whether C5 was appropriately inhibited.[49] In order to rule out if complement inhibition would be beneficial in patients with acute severe HF and cardiogenic shock due to MI, more clinical trials are needed.

The current study is of explorative character, however, on a well-defined cohort with close follow-up and careful plasma preparation, which is critical for accurate complement analysis. The low numbers of patients in the group of cardiogenic shock as well as the lack of blood samples before PCI are limitations of the present study. The major differences found between the groups, with statistical significance for all complement activation products and endothelial cell markers, however, increase the impact of the data because the risk of type I error can be regarded as small.

The patients included in this study represent a group of patients often excluded from clinical trials due to the severity of the disease. However, our results, consistently demonstrating an increased and persistent complement activation correlating to disease severity and endothelial cell activation, indicating that patients with advanced HF complicating large MI, may particularly benefit from therapy targeting complement activation. Our findings add new understanding to the inflammatory

profile in patients with acute severe HF, which can pave the way for new prognostic markers and targets for therapy.

Conflict of interest

None declared.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article.

Table S1. Spearman correlation analysis and linear regression analyses between peak values of sC5b-9 and various relevant variables.

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