# Complement activation products in acute heart failure: potential role in pathophysiology, response to treatment and impact on long-term survival

Marten Trendelenburg, MD[1,2*]; Fabio Stallone, MD[1,3,4*]; Kateryna Pershyna, MD[3,4]; Timo Eisenhut, MD[3,4,]; Raphael Twerenbold, MD[3,4,]; Karin Wildi, MD[3,4,5]; Denise Dubler, PhD[2]; Lucia Schirmbeck, MSc[2]; Christian Puelacher, MD[3,4]; Maria Rubini Gimenez, MD[3,4]; Zaid Sabti, MD[3,4]; Luca Osswald, MD[3,4]; Tobias Breidthardt, MD[1,3,4]; and Christian Mueller, MD[3,4]

[1]*Division of Internal Medicine,* University Hospital Basel, Switzerland*;* [2] *Laboratory for Clinical Immunology, Department of Biomedicine,* University Hospital Basel, Switzerland*;* [3]*Department of Cardiology;* [4]*Cardiovascular Research Institute Basel (CRIB) ,* University Hospital Basel, Switzerland*;* [5] *Department of Intensive Care Medicine,* University Hospital Basel, Switzerland

*both authors have contributed equally and should be considered as first author

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**Correspondence to:** Prof. Dr. Christian Müller**,** Department of Cardiology, University Hospital Basel**,** Petersgraben 4, CH-4031 Basel, Switzerland

E-mail: christian.mueller@usb.ch

**Abstract**

**Background:** Previous studies indicated a correlation between heart failure, inflammation and poorer outcome. However, the pathogenesis and role of inflammation in acute heart failure (AHF) is incompletely studied and understood. The aim of our study was to explore the potential role of innate immunity -quantified by complement activation products (CAP)- in pathophysiology, response to treatment and impact on long-term survival in AHF.

**Methods**: In a prospective study enrolling 179 unselected patients with AHF, plasma concentrations of C4d, C3a, and sC5b-9 were measured in a blinded fashion on the first day of hospitalization and prior to discharge. The final diagnosis including the AHF phenotype was adjudicated by two independent cardiologists. Long-term follow-up was obtained. Findings in AHF were compared to that obtained in 75 healthy blood donors (control group)

**Results**: Overall, concentrations of all three CAP were significantly higher in patients with AHF than in healthy controls (all $p<0.001$). In an age-adjusted subgroup analysis, significant differences could be confirmed for concentrations of C4d and sC5b-9, and these parameters further increased after 6 days of in-hospital treatment ($p<0.001$). In contrast, C3a levels in AHF patients did not differ from those of the control group in the age-adjusted subgroup analysis, and remained constant during hospitalisation. Concentrations of C4d, C3a and sC5b-9 were significantly higher when AHF was triggered by an infection as compared to other triggers ($p<0.001$). In addition, CAP levels significantly correlated with each other ($r=0.55$ to $0.76$), but did not predict death within 2 years.

**Conclusions:** Activation of complement with increased plasma levels of C4d and sC5b-9 at admission and increasing levels during AHF treatment seems to be associated with AHF, in particular when AHF was triggered by infection. However, complement activation products do not have a prognostic value in AHF

## Introduction

Acute heart failure (AHF) is the most common cause of hospital admission in patients older than 65 years of age and continues to have unacceptably high rates of mortality and morbidity. [1-6] Poor outcome in AHF may at least in part be due to incomplete understanding of the pathophysiology of AHF. In addition, incomplete understanding of AHF pathophysiology may have contributed to the fact that none of the currently available treatments is supported by an appropriately powered positive randomized controlled study and none of the recently completed phase III studies of novel drugs demonstrated clear clinically relevant benefit. [1, 2, 7-9]

More in-depth knowledge of pathogenic mechanisms being involved in AHF seem to be a prerequisite for more targeted and successful interventions. E.g. in acute myocardial infarction identification of thrombotic plaque rupture as its key pathophysiological hallmark has led to the enormous treatment successes in patients suffering from AMI by antiplatelet therapy, anticoagulation, statins, and early revascularization.[10]

The complement system is one of the key components of innate immunity. After activation it has at least three physiological functions: Defense against infections, bridging innate and adaptive immunity, and clearing apoptotic cells and immune

complexes. The complement system achieves that by the opsonisation of pathogens for phagocytes, the release of inflammatory mediators and the induction of cell lysis through the formation of the membrane attack complex. [11-21] On the other hand, inappropriate complement activation is pathogenic and may be involved in various autoimmune diseases. The complement system features more than 20 different serum proteins that are produced by a variety of cells. The interaction of these proteins constitutes a meticulously regulated cascade of activation steps (Figure 1). Finally, all activation steps converge on the complement component C3. In the heart, activated C3 (C3a) caused tachycardia, impairment of atrioventricular conduction, and left ventricular contractility. [11-21]

Pilot studies have suggested an activation of inflammatory pathways in chronic HF and regression after treatment as well as an association between inflammation and poorer outcome.[22-26] However, the pathogenic mechanisms leading to the activation of the immune system and to a poorer outcome are largely unknown. While several recent studies have examined complement components in patients with AMI, their possible role in heart failure in general and AHF in particular are still unclear.[11-21]

Considering the association between inflammation and CHF, poorer outcome and the incomplete understanding of the pathogenic mechanisms of inflammation in CHF we aimed to explore the activation of the innate immunity in AHF determining levels of complement activation products (CAP) C4d, C3a, and sC5b-9 in post hoc analysis of a population of AHF patients that were recruited in the context of a prospective study. We postulated that a higher degree of complement activation will be associated with poorer outcome in these patients. In addition, for a better understanding of the pathogenesis of complement-associated inflammation in AHF, we evaluated predictors of low and high CAP levels in this AHF population.

## Methods

### *Study design and population*

Unselected AHF patients presenting to the ED of the University Hospital Basel (Switzerland) were enrolled in this prospective observational study. More than 18 year-old men and women, presenting with acute dyspnea as the main symptom and diagnosed as having AHF were recruited. All patients underwent a clinical assessment that included medical history, physical examination, 12-lead ECG, continuous ECG rhythm monitoring, pulse oximetry, standard blood test, and chest radiography. Treatment of patients was left to discretion of the attending physician. The final diagnosis, phenotype of heart failure and trigger of the acute decompensation was then adjudicated using all medical records pertaining to the patients (including results of standard investigations, response to therapy and B-type natriuretic peptide (BNP) values) by two independent cardiologists according to the current guidelines.[1]

The study was conducted according to the principles of the Declaration of Helsinki and approved by the local ethics committee. Written informed consent was obtained from all participating patients. While recruitment was independent of renal function, patients with terminal kidney failure requiring chronic hemodialysis were excluded. For this analysis, patients with AHF triggered by ST-elevation MI and patients with cardiogenic shock were also excluded.

### *Follow-up*

The primary prognostic endpoint was all cause-mortality during follow-up. After 3, 12 and 24 months, patients were contacted by phone or by letter and were interviewed by trained researchers blinded to the results of laboratory testing. Information about all cardiovascular events since presentation at the emergency departement until death

was obtained by the patient's family physician, the hospital's diagnosis registry or the national registry on mortality.

### *Control group*

In order to validate the measurements of the levels of complement activation products (CAP), we also determined CAP levels in 75 healthy blood donors. This age and gender unadjusted population then was used as a control group to compare levels of CAP in AHF with those in healthy subjects.

### *Biochemical Analysis*

For all the patients, venous blood samples were collected in tubes containing potassium EDTA. BNP was measured immediately using the Abbott Architect assay; otherwise, samples were centrifuged and frozen at -80°C until later analysis in a dedicated core laboratory. Plasma levels of three complement activation products (C4d, C3a, sC5b-9) were measured by enzyme-linked immunosorbent assays from samples taken on day 1 of hospitalization as well as one the day prior to discharge and using commercially available kits following the manufacturer's instructions (C3a Plus MicroVue, C4d MicroVue, SC5b-9 Plus MicroVue, all Quidel, San Diego). In short, the C4d assay measures all C4d-containing fragments of C4 present in human plasma. In contrast, the C3a and sC5b-9 assays were designed to specifically detect complement activation products (CAP) with the C3a assay detecting a neo-epitope on C3a (C3a-desArg) that is generated during complement activation, and the sC5b-9 immunoassay measuring the concentration of soluble terminal complement complexes.

## Statistical Analysis

Categorical variables are expressed as numbers and percentages, and continuous variables are expressed as medians and interquartile range.

To evaluate a possible activation of the complement cascade in AHF, we compared levels of CAP in AHF patients with a control group of 75 healthy blood donors. Median values of CAP levels for AHF patients and the control group were compared by Mann-Whitney U tests, and chi-squared tests was used to compare the gender distribution. Due to differences in age between patients and healthy controls, we performed an age-related subgroup analysis only for those individuals with age of 60 to 69 years and 70 to 79 years. Numbers of patients for other groups of age were considered to be too low for an explanatory power. To screen predictors for patients with levels of all three complement activation products being in the higher or lower tertile respectively, we primarily performed univariate logistic regression. We assumed that the class of patients with levels of complement activation products only in the highest or lowest tertile had the highest or lowest activation of the complement cascade. To the multivariate model we included age, sex, BMI and all variables associated with p-values lower than 0.1 in the univariate analysis for lower or higher tertile. Correlation analyses were performed using Spearman rank correlation test. Receiver operating characteristic (ROC) and Kaplan Meier curves were constructed to assess prognostic value of complement activation products at admission with regard to 730-day mortality. Time to death during follow-up was compared between tertiles of complement activation products on admission and analysed by a log-rank test. COX-Regression analysis were ruled to asses a multivariable adjusted prognostic value regarding complement activation products at discharge with regard to 730-day mortality. Variables with p-value <0.1 and sex were included in the multivariate analysis. The multivariate model was the reference model to test the prognostic value of each

complement activation product at discharge as continous variable. All hypothesis testing was two-tailed and a P-value of 0.05 or below was considered statistically significant. All statistical analyses were performed using SPSS for Windows 22.0 (IBM) and MedCalc 11.2.1.0 (MedCalc Software).

## Results

### *Characteristics of Patients*

The baseline characteristics of the 179 AHF patients are shown in Table 1.

### *Complement activation products (CAP) in AHF versus controls and response to treatment*

Concentrations of CAP were significantly higher (all p<0.001; Table 2) in patients with AHF as compared to a gender and age unadjusted control group (median age 56 years (IQR 47-68 years), 23% women (p<0.001 for comparisons with the AHF group)). A subgroup analysis for patients with age between 60 and 69 years as well as between 70 and 79 years could confirm differences for levels of C4d and sC5b-9 (all p<0.001 and p=0.002 for sC5b-9 in individuals with age of 70-79 years), whereas no difference in levels of C3a in AHF compared to the control group could be found (p= 0.432 and p=0.061, respectively). Interestingly, while concentrations of C4d and sC5b-9 further increased during hospitalisation (p<0.001), concentrations of C3a remained constant (p=ns; Figure 2 (a) to (c)).

Concentrations of CAP significantly correlated with each other (r=0.55 to 0.76). Spreading plots with best-fitted lines between the 3 different CAP are shown in Figure 3 (a) to (c). The correlations were moderate to high: r= 0.64, p<0.001 for correlation between C3a and C4d; r= 0.76, p<0.001 for C3a and sC5b-9; r= 0.64, p<0.001 for C4d and sC5b-9). C4d, C3a and sC5b-9 had a weak correlation with CRP (r= 0.20, p=0.007, r= 0.004, p=0.963; r= 0.155, p= 0.40) and a low inverse correlation with BNP (r= -0.20, p= 0.007; r= -0.18, p= 0.015 and r = -0.30, p<0.001). Levels of C4d and C3a at

discharge showed no correlation with BNP at discharge (p=ns), but levels of sC5b-9 had a small negative correlation with BNP at discharge (r=-0.154, p= 0.040). No correlation between CRP and complement activation products was found at discharge (p=ns)

### *Complement activation products in patients with AHF and infectious trigger*

Concentrations of C4d, C3a and sC5b-9 were significantly higher when AHF was triggered by an infection as compared to other triggers (p<0.001, Table 3). Of note, levels of C4d and sC5b-9 were still significantly higher in patients without an infectious trigger than in the control group (p<0.001) while there was no difference for levels of C3a between patients with AHF but without infectious trigger and controls (p=0.160).

### *Complement activation products in patients with AHF and coronary artery disease with or without ischemic trigger*

Plasma concentrations for the three CAP were similar in patients with or without coronary artery diesease (CAD) (Online only Table 1). Patients with an ischemic trigger of AHF had slightly, but statistically not significant, higher concentrations of C4d and C3a when compared to patients without an ischemic cause. (Online Only Table 2). Even after exclusion of patients with an infectious trigger there was no statistically significant difference (data not shown).

### *Predictors of elevated or low complement activation products at AHF presentation*

Univariate logistic regressions for the association of patients with all CAP in the lower and higher tertile are shown in the online only table 3. After multivariate adjustement (Table 4), it could be observed that patients with complement activation products in the lower tertile had less often AHF associated with infection. Other variables did not reach statistical significance. Patients in the higher tertile were associated more often with lower BNP (OR 0.92 (0.85-1.00), p-value 0.050) and lower ALAT levels (OR 0.94 (0.90-0.99), p-value 0.011).

### *Complement activation products and long-term mortality in AHF*

There were 66 events in a 2-year follow up. 730-day follow ups were completed in 43/113 (38%) patients without event and 1-year follow-up was completed in 112/113 (99%).

Concentrations at admission of all three investigated complement activation products did not predict death within 2 years. Prognostic accuracy as quantified by the AUC is shown in Figure 4, Kaplan Meier survival curves in AHF patients classified according to tertile of each CAP are shown in Figure 5 (a) to (c). Mortality rate among different tertiles of CAPs was the same (Log rank =ns).

In a multivariate analysis liver disease, eGFR and CRP at discharge were associated with mortality. Levels of complement activation products at hospital discharge were not associated with long-term mortality (table 5).

## Discussion

This prospective study explored the possible pathogenic, diagnostic and prognostic role of complement factors in AHF by measuring plasma C4d, C3a, and sC5b-9 levels.

We report 4 major findings. First, when compared to an unadjusted control group of healthy blood donors, plasma concentrations of all three complement activation products (CAP) were significantly higher in patients with AHF. However, in a sub-group analysis for patients with 60 to 69 years and 70 to 79 years of age, differences could only be confirmed for levels of C4d and sC5b-9. Second, higher concentrations of C4d, C3a and sC5b-9 at admission were more often associated with an infection as trigger of AHF, as well as with lower BNP and ALAT levels. Patients with an ischemic trigger had slightly, but statistically not significant, higher concentrations of C4d and C3a at admission. Third, levels of C4d and sC5b-9 continued to rise during the hospitalisation, whereas levels of C3a remained stable, suggesting ongoing activation of the complement cascade in AHF via the classical and/or lectin pathways that is not rapidly influenced by the treatment of acute heart failure. Fourth, complement activation products at admission and discharge were not associated with long-term mortality in AHF.

Our findings corroborate and extend findings from basic as well as pre-clinical and clinical models [11-21] suggesting that complement activation is mechanistically involved in adverse cardiac healing and remodeling after AMI. Complement inhibition consistently attenuated leukocyte recruitment following AMI highlighting the critical role of the complement cascade in triggering inflammation in the ischemic myocardium.

However, the possible role of complement factors in AHF or chronic HF is still emerging. Recently, Gombos et al. examined stable outpatients with chronic HF and found an association between activated complement C3a and mortality or rehospitalisation due to progression of heart failure.[16] Aukrust et al. found complement activation with chronic heart failure, while treatment with intravenous immunoglobulin seemed to reduce complement activation and increased left ventricular function during a 5-month follow-up period.[14] In a recent study, Silva et al. reported on lower levels of

C3c and C4c being more often associated with high levels of BNP at admission for AHF, suggesting an activation of the complement cascade in AHF. In a multivariate analysis, low C3c levels at discharge were independently associated with the 6-month mortality.[27] However, while we observed some association between complement activation products and BNP levels in our study as well, we could not find any association between complement activation products and mortality. These differences might be due to the different parameters being measured (C3c and C4c versus C4d and C3a), different methods used for their quantification (nephelometry versus enzyme immunoassays) as well as differences in time and character of storage of samples prior to analysis.

While this is one of the first studies to explore complement activation products in AHF, it is important to acknowledge several limitations. First, our study is moderate in size and recruited patients from a single institution, rendering it susceptible to inclusion bias. In this context, it is reassuring to note that baseline characteristics of this study were similar to e.g. large international AHF registries.[28-30] Second, we measured only three activation products of the complement cascade allowing only limited conclusions on more subtle alterations in the complement system. Third, we cannot exclude effects due to the period of freezing at -80˚C on complement split product levels that could only be minimized by e.g. determination of markers of complement activation in samples cooled immediately after blood collection. However, significant differences between samples of AHF patients having undergone the same handling procedures but having had different triggers of disease make it rather unlikely that this issue was a major confounder. In addition, control samples had also been stored at -80°C prior to use. Forth, we cannot comment on the possible role of complement activation products in terminal kidney failure requiring dialysis, as those patients were excluded from our study. Fifth, Patients stratification in tertiles is arbitrary and based on the

hypothesis that patients with complement activation products in the higher or lower tertile have a higher or lower complement activation. Sixth, the control group used for comparison of CAP levels was a cohort of healthy patients unadjusted to the baseline characteristics of the AHF cohort. Although our observation of increased complement activation is well in line with findings of previous studies [11, 13, 24] further studies will be required to determine the diagnostic as well pathogenic specificity of the observed complement activation in this acute heart failure cohort, in particular in relation to age, gender and comorbidity of the patients. In order to address this issue in our cohort, we performed an age-adjusted subgroup analysis in individuals with age of 60 to 69 and 70 to 79 years respectively. In both subgroups we could confirm increased levels of C4d and sC5b-9. Interestingly, these two parameters further increased during the first days of in-hospital care, and were also higher in AHF patients without infectious trigger. This observation might point to a prominent role of the classical and/or lectin pathway of complement in AHF and pathogenic mechanisms that either have a delayed response to treatment or require more profound changes than those being achieved during the first days of acute medical care. Last, although our data point to pathogenic role of complement activation AHF, our data do not allow dissecting more detailed mechanisms, in particular with regard to the question whether complement activation occurred locally or systemically. Our primary hypothesis was, that heart failure induces complement activation that can be quantified in peripheral blood, but it is also possible that complement activation occurred more systemically being associated with or even leading to acute heart failure. The dissection of these mechanisms will require a more experimental approach.

In conclusion, complement activation products, in particular C4d and sC5b-9 suggesting activation via the classical and/or lectin pathway, seems to be associated

with AHF, as their levels are substantially increased at AHF presentation to the ED and changes during the AHF treatment. However, CAP did not have a prognostic value in AHF

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## Disclosures

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# Tables and figures

**Table 1.** Baseline characteristics of the 179 patients with AHF

| | |
|---|---|
| Age, ys. – median (IQR) | 79 (70-84) |
| BMI, kg/cm$^2$ - median (IQR) | 26(23-30) |
| Sex, female (%) | 80 (45) |
| NYHA class III-IV at admission (%) | 168 (95) |
| Cardiovascular risk factors – no (%) | |
| Hypertension | 136 (76) |
| Diabetes | 57 (32) |
| Dyslipidemia | 70 (41) |
| Smoking | 110 (61) |
| Medical history – (%) | |
| Coronary artery disease | 102 (57) |
| COPD | 39 (22) |
| Renal failure | 76 (43) |
| Malignant disease | 34 (19) |
| Atrial fibrillation | 64 (36) |
| Clinical findings | |
| Systolic blood pressure – mmHg – median (IQR) | 137 (122-160) |
| Diastolic blood pressure – mmHg – median (IQR) | 84 (72-96) |
| Heart rate – median (IQR) | 89 (75-111) |
| Oxygen saturation – % (IQR) | 96 (94-98) |
| ECG findings – (%) | |
| Sinus rhythm | 88 (49) |
| Bundle branch block | 129 (72) |
| Radiographic findings – no (%) | |
| Cardiomegalie | 105 (59) |
| Apical redistribution | 79 (44) |
| Interstitial edema | 49 (27) |
| Alveolar edema | 9 (5) |
| Laboratory findings – median (IQR) | |
| BNP (ng/l) | 891 ( 476-1786) |
| Creatinin (mg/dl) | 104 (76-145) |
| eGFR (mL/min/1.73 m$^2$) | 47 (31-73) |
| CRP (mg/dl) | 12 (4-40) |
| Haemoglobin (g/l) | 129 (116-143) |
| Medication – no (%) | |
| ACE or AT2 inhibitor | 110 (62) |
| Calcium antagonist | 59 (34) |
| Diuretic | 131 (73) |
| Betablocker | 105 (59) |
| Statin | 60 (34) |
| Antiaggregants[a] | 76 (43) |
| Anticoagulant[b] | 73 (41) |

BMI, Body mass index; NYHA, New York heart association functional classification; COPD , Chronic obstructive pulmonary disease; BNP, B-Type natriuretic peptide; eGFR, estimated glomerular filtration rate measured with the Crockoft-Gault Equation; C-reactive protein per; ACE, angiotensin-converting-enzyme inhibitor; AT2, Angiotensin II receptor; [b]Aspirin or Clopidogrel; [c]Phenprocoumon

**Table 2.** Levels of complement activation products (C4d, C3a, sC5b-9) in a control group of healthy individuals and patients with AHF. Subgroup analysis were performed for patients with age between 60 and 69 years (n=15 in the control group, n=31 in the AHF group) and 70-79 years (n= 15 in the control group, n=53 in the AHF group).

| | Control group (n=75) | AHF (n=179) | p-value |
|---|---|---|---|
| C3a baseline (ng/ml) | 312 (187-435) | 440 (232-715) | <0.001 |
| 60-69 years | 354 (218-406) | 354 (212-705) | 0.432 |
| 70-79 years | 312 (219-655) | 535 (336-809) | 0.061 |
| C4d baseline (µg/ml) | 1.75 (0.58-2.77) | 6.73 (3.51-10.76) | <0.001 |
| 60-69 years | 0.74 (0.00-1.13) | 6.20 (2.92-12.70) | <0.001 |
| 70-79 years | 1.51 (0.57-4.60) | 7.58 (4.55-11.33) | <0.001 |
| sC5b-9 baseline (ng/ml) | 184 (154-252) | 389 (287-540) | <0.001 |
| 60-69 years | 180 (167-265) | 326 (287-463) | <0.001 |
| 70-79 years | 234 (172-373) | 409 (302-574) | 0.002 |

**Table 3.** Levels of complement activation products (C4d, C3a, sC5b-9) in patients with infectious as trigger of acute heart failure

| | Infection (n=84) | Other triggers (n=95) | p-value |
|---|---|---|---|
| C3a baseline (ng/ml) | 562 (343-807) | 347 (186-639) | <0.001 |
| C3a day 6 (ng/ml) | 569 (313-860) | 364 (199-624) | <0.001 |
| C4d baseline (µg/ml) | 7.79 (4.12-12.34) | 5.71 (3.30-9.64) | <0.001 |
| C4d day 6 (µg/ml) | 9.14 (5.29-13.12) | 7.27 (4.44-12.51) | <0.001 |
| sC5b-9 baseline (ng/ml) | 428 (345-582) | 342 (263-485) | <0.001 |
| sC5b-9 day 6 (ng/ml) | 481 (346-630) | 390 (273-576) | <0.001 |

Other triggers include dietary indiscretion, medication malcompliance, uncontrolled hypertension, myocardial ischemia, arrhythmia, progressive valvular disease, anemia, thyroid disorders, pulmonary causes

**Online Table 1.** Levels of complement activation products (C4d, C3a, sC5b-9) in patients with coronary artery disease

| | CAD (n=102) | Other cardiopathy (n=77) | p-value |
|---|---|---|---|
| C3a baseline (ng/ml) | 416 (242-734) | 457 (222-705) | 0.690 |
| C3a day 6 (ng/ml) | 513 (279-749) | 411 (225-779) | 0.510 |
| C4d baseline (µg/ml) | 6.63 (3.33-10.69) | 6.94 (3.94-12.16) | 0.903 |
| C4d day 6 (µg/ml) | 8.65 (4.62-13.86) | 8.57 (4.89-11.87) | 0.354 |
| sC5b-9 baseline (ng/ml) | 383 (302-532) | 395 (281-541) | 0.720 |
| sC5b-9 day 6 (ng/ml) | 413 (294-590) | 456 (311-635) | 0.612 |

CAD, coronary artery disease

**Online Table 2.** Levels of complement activation products (C4d, C3a, sC5b-9) in patients with acute ischemia as trigger of acute heart failure

| | Acute Ischemia (n=13) | Other triggers (n=166) | p-value |
|---|---|---|---|
| C3a baseline (ng/ml) | 475 (233-644) | 434 (232-715) | 0.945 |
| C3a day 6 (ng/ml) | 582 (279-802) | 448 (253-768) | 0.794 |
| C4d baseline (µg/ml) | 9.64 (5.05-16.36) | 6.51 (3.48-10.24) | 0.051 |
| C4d day 6 (µg/ml) | 10.40 (6.07-18.48) | 8.57 (4.80-12.54) | 0.236 |
| sC5b-9 baseline (ng/ml) | 375 (332-610) | 390 (282-532) | 0.371 |
| sC5b-9 day 6 (ng/ml) | 503 (320-837) | 442 (305-592) | 0.456 |

Other triggers include dietary indiscretion, medication malcompliance, uncontrolled hypertension, infection, arrhythmia, progressive valvular disease, anemia, thyroid disorders, pulmonary causes

**Online Table 3.** Univariate logistic regression for the evaluation of predictors of low (1st tertile) and high (3rd tertile) levels of complement activation products at admission, respectively. In the 1st tertile (n=30) patients are included who had only levels of complement activation products (C4d, C3a and sc59b) being in the lowest tertile. In the 3rd tertile (n=30) patients are included who had only levels of complement activation products being in the highest tertile.

| | **1st tertile vs 2nd and 3rd tertiles** | | **3rd tertile vs 1st and 2nd tertiles** | |
|---|---|---|---|---|
| | **OR (95%CI)** | **p-value** | **OR (95%CI)** | **p-value** |
| Age, ys. | 0.99 (0.96-1.03) | 0.785 | 0.98 (0.95-0-1.02) | 0.258 |
| BMI, kg/cm$^2$ | 0.99 (0.93-1.07) | 0.969 | 1.03 (0.97-1.10) | 0.319 |
| Reduced LVEF | 1.67 (0.69-4.03) | 0.255 | 0.52 (0.20-1.34) | 0.179 |
| Sex, female | 0.56 (0.25-1.29 | 0.174 | 0.79 (0.36-1.77) | 0.571 |
| NYHA class | 0.76 (0.38-1.48) | 0.415 | 0.98 (0.50-1.89) | 0.940 |
| Cardiovascular risk factors | | | | |
| Hypertension | 1.05 (0.42-2.64) | 0.923 | 0.84 (0.35-2.06) | 0.710 |
| Diabetes | 0.37 (0.14-1.03) | 0.058 | 0.90 (0.39-2.12) | 0.812 |
| Dyslipidemia | 1.45 (0.65-3.23) | 0.364 | 2.03 (0.91-4.54) | 0.086 |
| Active Smoking | 0.20 (0.12-1.55) | 0.202 | 1.44 (0.56-3.71) | 0.450 |
| Medical history | | | | |
| Previous AHF | 1.13 (0.51-2.47) | 0.765 | 1.85 (0.82-4.15) | 0.137 |
| Coronary artery disease | 0.82 (0.37-1.80) | 0.617 | 1.33 (0.60-2.92) | 0.485 |
| COPD | 0.92 (0.35-2.44) | 0.862 | 2.05 (0.87-4.85) | 0.102 |
| Renal failure | 1.23 (0.56-2.70) | 0.610 | 1.04 (0.47-2.30) | 0.915 |
| Malignant disease | 0.61 (0.20-1.88) | 0.390 | 1.38 (0.54-3.53) | 0.508 |
| Hypertensive heart disease | 0.50 (0.18-1.41) | 0.190 | 1.05 (0.44-2.51) | 0.913 |
| Atrial fibrillation | 0.99 (0.44-2.25) | 0.989 | 0.57 (0.24-1.37) | 0.210 |
| Liver Disease | 0.34 (0.43-2.73) | 0.313 | 1.26 (0.33-4.76) | 0.734 |
| Clinical findings | | | | |
| Systolic blood pressure – mmHg – median (IQR) | 0.99 (0.98-1.00) | 0.138 | 1.00 (0.98-1.01) | 0.911 |
| Diastolic blood pressure – mmHg – median (IQR) | 0.99 (0.97-1.01) | 0.492 | 0.99 (0.97-1.01) | 0.485 |
| Heart rate – median (IQR) | 1.00 (0.99-1.02) | 0.629 | 0.99 (0.97-1.00) | 0.131 |
| Oxygen saturation – % (IQR) | 1.08 (0.96-1.22) | 0.199 | 0.95 (0.87-1.04) | 0.252 |
| Hepatojugular reflux – no (%) | 0.51 (0.18-1.45) | 0.209 | 0.88 (0.36-2.16) | 0.784 |
| Jugular distension – no (%) | 1.53 (0.65-3.61) | 0.330 | 0.44 (0.18-1.04) | 0.062 |
| Leg edema – no (%) | 0.76 (0.33-1.77) | 0.535 | 0.64 (0.28-1.46) | 0.289 |
| Sinus rhythm | 0.89 (0.40-1.95) | 0.765 | 1.69 (0.76-3.76) | 0.196 |
| Bundle branch block | 1.63 (0.71-3.74) | 0.245 | 0.24 (0.07-0.84) | 0.025 |
| Radiologic signs of leftventricular AHF[a] | 0.66 (0.30-1.44) | 0.293 | 0.47 (0.21-1.05) | 0.065 |
| Laboratory findings | | | | |
| BNP (ng/l) | 1.02 (1.00-1.04) | 0.108 | 0.90 (0.83-0.97) | 0.006 |

| | | | | |
|---|---|---|---|---|
| eGFR (mL/min/1.73m$^2$) | 1.01 (0.99-1.02) | 0.479 | 1.00 (0.99-1.01) | 0.510 |
| CRP (mg/dl) | 0.98 (0.96-1.00) | 0.049 | 1.00 (0.99-1.01) | 0.427 |
| Bilirubin (µmol/L) | 1.01 (0.98-1.04) | 0.588 | 1.01 (0.98-1.03) | 0.735 |
| ALAT (U/l) | 1.00 (1.00-1.01) | 0.283 | 0.96 (0.93-0.99) | 0.028 |
| ASAT (U/l) | 1.00 (1.00-1.01) | 0.617 | 0.99 (0.97-1.01) | 0.236 |
| INR | 1.05 (0.80-1.38) | 0.731 | 1.11 (0.86-1.44) | 0.427 |
| Albumin (g/l) | 1.05 (0.96-1.14) | 0.284 | 0.96 (0.89-1.04) | 0.307 |
| Haemoglobin (g/l) | 1.01 (0.99-1.03) | 0.303 | 1.00 (0.98-1.02) | 0.745 |
| Leucocyte (x $10^9$/l) | 0.92 (0.81-1.04) | 0.183 | 1.05 (0.94-1.18) | 0.377 |
| Thrombocytes (x $10^9$/l) | 1.00 (1.00-1.01) | 0.358 | 1.00 (1.00-1.01) | 0.379 |
| Medication | | | | |
| ACE or AT2 inhibitor | 2.93 (1.13-7.59) | 0.027 | 1.10 (0.49-2.48) | 0.817 |
| Calcium Antagonist | 0.99 (0.42-2.36) | 0.982 | 0.82 (0.35-1.93) | 0.654 |
| Diuretic | 0.34 (0.15-0.76) | 0.009 | 1.57 (0.60-4.11) | 0.359 |
| Betablocker | 0.66 (0.30-1.44) | 0.293 | 1.07 (0.48-2.38) | 0.870 |
| Antiaggregants[b] | 0.75 (0.33-1.68) | 0.483 | 0.75 (0.33-1.68) | 0.483 |
| Anticoagulant[c] | 1.13 (0.51-2.51) | 0.755 | 1.85 (0.84-4.06) | 0.129 |
| Statin | 1.17 (0.50-2.73) | 0.726 | 0.96 (0.42-2.21) | 0.923 |

BMI, Body mass index; NYHA, New York heart association functional classification; COPD , Chronic obstructive pulmonary disease; BNP, B-Type natriuretic peptide; eGFR, estimated glomerular filtration rate measured with the Crockoft-Gault Equation; C-reactive protein per; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; ACE, angiotensin-converting-enzyme inhibitor; AT2, Angiotensin II receptor; INR, international normalized ratio; [a] Radiologic signs for apical redistribution or interstitial or alveolar oedema; [b]Aspirin or Clopidogrel; [c]Phenprocoumon; OR for continue variable are per increase in 1 Unit, for BNP OR are per increase in 100ng/l

**Table 4.** Multivariate logistic regression for the evaluation of predictors of low (1st tertile) and high (3rd tertile) levels of complement activation products at admission, respectively. In the 1st tertile (n=30) patients are included who had only levels of complement activation products (C4d, C3a and sc59b) being in the lowest tertile. In the 3rd tertile (n=30) patients are included who had only levels of complement activation products being in the highest tertile.

| | **1st tertile vs 2nd and 3rd tertiles** | | **3rd tertile vs 1st and 2nd tertiles** | |
|---|---|---|---|---|
| | **OR (95%CI)** | **p-value** | **OR (95%CI)** | **p-value** |
| Age, ys. – median (IQR) | 1.03 (0.97-1.09) | 0.320 | 0.97 (0.92-0-1.02) | 0.227 |
| BMI, kg/cm$^2$ - median (IQR) | 1.00 (0.89-1.13) | 0.972 | 1.04 (0.93-1.15) | 0.506 |
| Sex, female (%) | 0.32 (0.09-1.14) | 0.079 | 1.05 (0.37-3.01) | 0.929 |
| Association with infection | 0.23 (0.06-0.93) | 0.039 | 2.17 (0.71-6.60) | 0.174 |
| Diabetes | 0.89 (0.22-3.47) | 0.857 | 0.50 (0.14-1.80) | 0.290 |
| Dyslipidemia | 2.60 (0.84-8.10) | 0.099 | 1.28 (0.44-3.73) | 0.651 |
| Jugular distension – no (%) | 1.19 (0.39-3.68) | 0.869 | 0.90 (0.29-2.81) | 0.859 |
| Bundle branch block | 0.70 (0.20-2.88) | 0.758 | 0.49 (0.12-2.06) | 0.328 |
| Radiologic signs of congestion | 0.51 (0.16-1.68) | 0.267 | 0.59 (0.19-1.82) | 0.359 |
| BNP (100 ng/l) | 1.03 (0.99-1.07) | 0.105 | 0.92 (0.85-1.00) | 0.050 |
| CRP (mg/dl) | 0.98 (0.94-1.01) | 0.163 | 1.00 (0.99-1.01) | 0.720 |
| ALAT (U/l) | 1.00 (1.00-1.01) | 0.487 | 0.94 (0.90-0.99) | 0.011 |
| ACE or AT2 inhibitor | 3.11 (0.86-11.30) | 0.084 | 2.26 (0.74-6.91 ) | 0.154 |
| Diuretic | 0.58 (0.17-1.98) | 0.381 | 1.81 (0.52-6.35) | 0.353 |

BMI, Body mass index; BNP, B-type Natriuretic Peptide; CRP, C-reactive protein; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; ACE, angiotensin-converting-enzyme inhibitor; AT2, Angiotensin II receptor;
Multivariate analysis included only patients without missing data (n= 151), 1st tertile n=23, 3rd tertile n=27. OR for continue variable are per increase in 1 Unit, for BNP OR are per increase in 100ng/l

**Table 5.** Univariate and multivariate Cox-regression analysis for 730 days mortality

| | Univariate Analysis | | Multivariate Analysis | |
|---|---|---|---|---|
| | **HR (95%CI)** | **p-value** | **HR (95%CI)** | **p-value** |
| Age, ys. | 1.05 (1.02-1.08) | 0.001 | 1.02 (0.99-1.06) | 0.207 |
| Sex, female | 0.94 (0.58-1.54) | 0.942 | 0.87 (0.50-1.51) | 0.618 |
| Ischemic heart disease | 1.15 (0.70-1.89) | 0.580 | | |
| Reduced LVEF | 1.00 (0.57-1.76) | 0.988 | | |
| Hypertension | 1.54 (0.81-2.95) | 0.189 | | |
| Diabetes | 0.84 (0.49-1.43) | 0.523 | | |
| Malignant Disease | 1.94 (1.12-3.32) | 0.012 | 1.48 (0.81-2.70) | 0.208 |
| Liver disease | 1.95 (0.93-4.11) | 0.079 | 2.50 (1.05-5.92) | 0.038 |
| Sinus Rhythm | 1.18 (0.73-1.91) | 0.499 | | |
| Bundle branch block | 0.78 (0.45-1.38) | 0.394 | | |
| ACE or AT2 inhibitor | 0.39 (0.22-0.67) | 0.001 | 0.60 (0.31-1.17) | 0.135 |
| Betablocker | 0.55 (0.33-0.90) | 0.018 | 0.69 (0.39-1.21) | 0.195 |
| Aspirin | 0.70 (0.41-1.21) | 0.200 | | |
| Clopidogrel | 1.57 (0.78-3.18) | 0.205 | | |
| Anticoagulant | 0.67 (0.41-1.10) | 0.115 | | |
| Statin | 0.72 (0.43-1.20) | 0.202 | | |
| Digoxin | 0.76 (0.31-1.90) | 0.562 | | |
| Spironolactone | 0.73 (0.35-1.52) | 0.395 | | |
| BNP (100 ng/l) | 1.03 (1.01-1.05) | 0.001 | 1.02 (1.00-1.04) | 0.124 |
| eGFR | 0.98 (0.97-0.99) | <0.001 | 0.99 (0.97-1.00) | 0.026 |
| CRP (mg/dl) | 1.01 (1.01-1.02) | <0.001 | 1.01 (1.01-1.02) | <0.001 |
| Albumin (g/l) | 0.89 (0.85-0.94) | <0.001 | 0.96 (0.90-1.03) | 0.246 |
| Haemoglobin (g/l) | 0.98 (0.97-0.99) | 0.015 | 1.00 (0.98-1.01) | 0.589 |
| C3a | 1.00 (1.00-1.00) | 0.197 | 1.00 (1.00-1.00) | 0.699 |
| C4d | 0.99 (0.95-1.03) | 0.598 | 0.99 (0.95-1.03) | 0.540 |
| sC5b-9 | 1.00 (1.00-1.00) | 0.817 | 1.00 (1.00-1.00) | 0.717 |

Reduced LVEF, Leftventricular ejection fraction lower than 45%; BNP, B-type Natriuretic Peptide; eGFR, estimated glomerular filtration rate measured with the Crockoft-Gault Equation; CRP, C-reactive protein;
HR for C3a, C4d, sc5b-9 in the multivariate model were obtained adding separately each complement activated product to the multivariate model.
HR for continue variable are per increase in 1 Unit, for BNP OR are per increase in 100ng
Multivariate analysis included only patients without missing data (n=169)

**Figure 1.** Schematic description of the complement pathways.

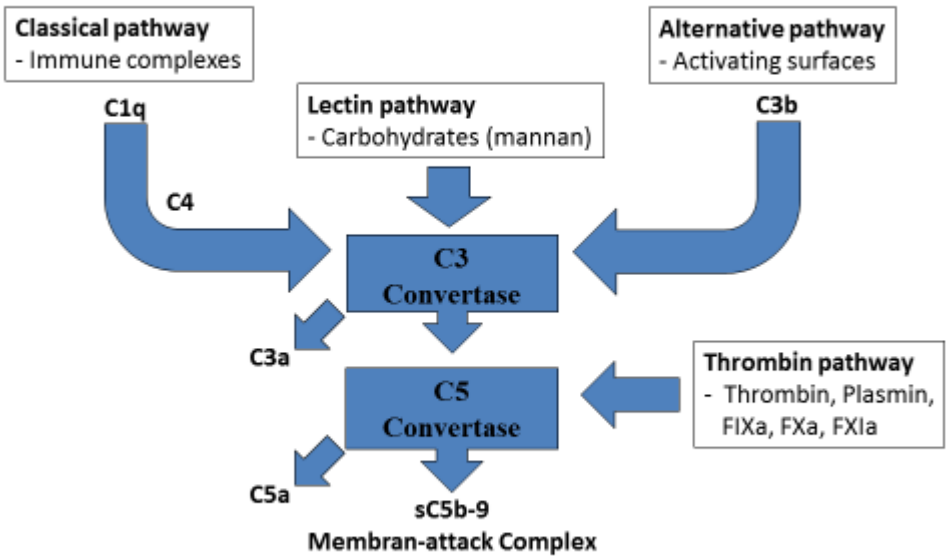
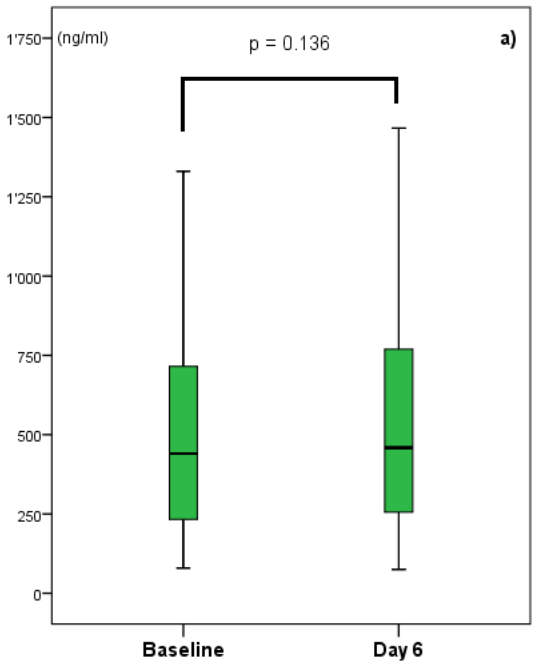
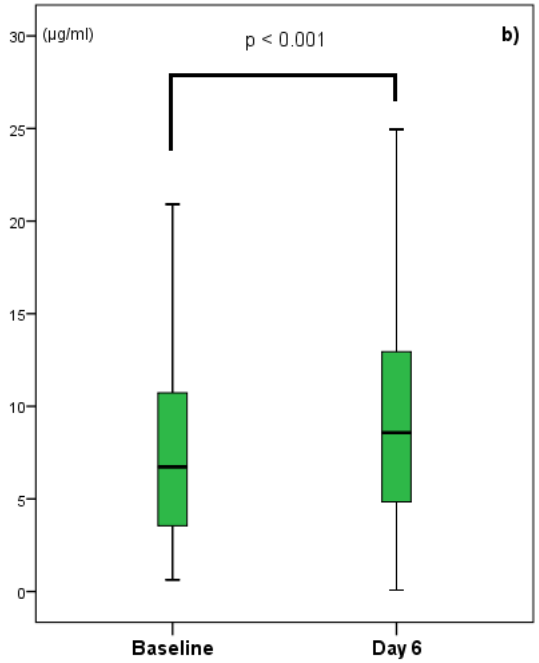


**Figure 2 (a),(b),(c).** Levels of complement activation products (CAP) in plasma (C3a (a), C4d (b), sC5b-9 (c)) at day 1 and day 6



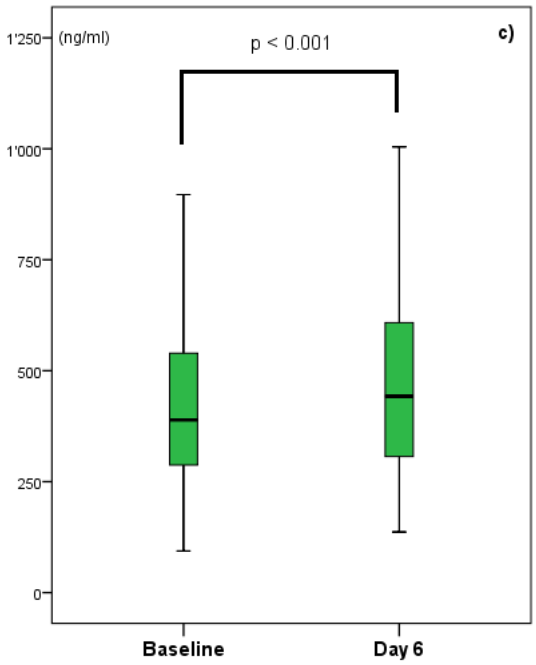
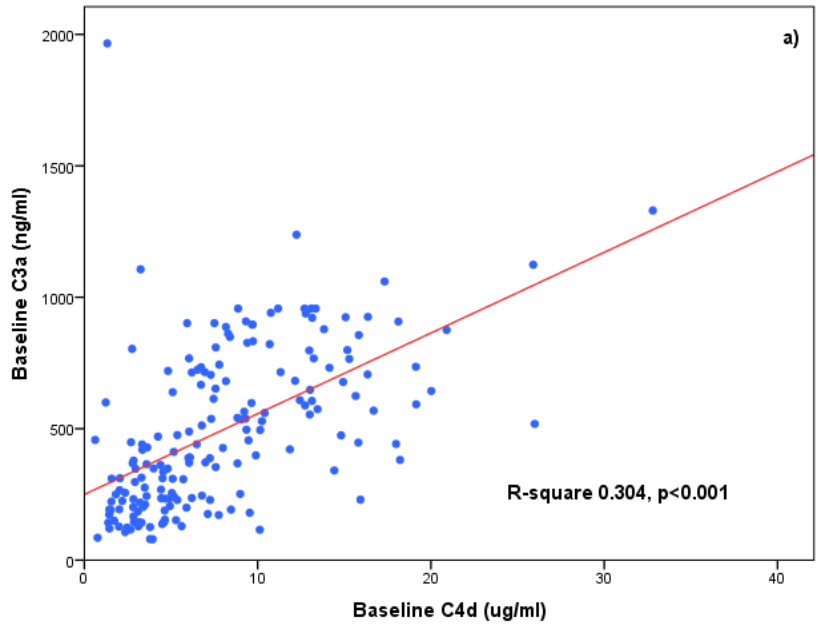
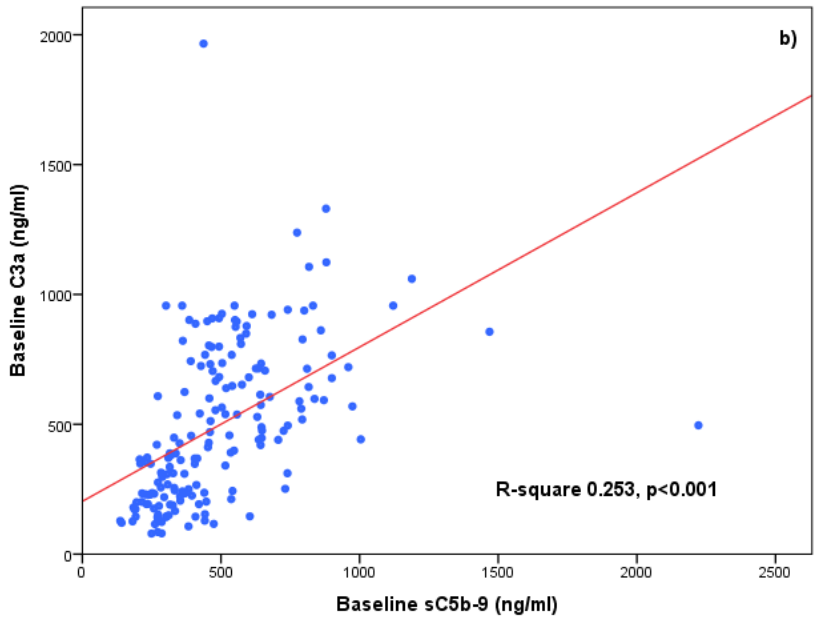



**Figure 3 (a),(b),(c).** Spreading plots with best fitting line for different CAPs. C3a and C4d (a, C3a and sC5b-9 (b), C4d and sC5b-9 (c)


a)
Baseline C3a (ng/ml)
2000
1500
1000
500
0
0
10
20
30
40
Baseline C4d (ug/ml)
R-square 0.304, p<0.001


b)
Baseline C3a (ng/ml)
2000
1500
1000
500
0
0
500
1000
1500
2000
2500
Baseline sC5b-9 (ng/ml)
R-square 0.253, p<0.001

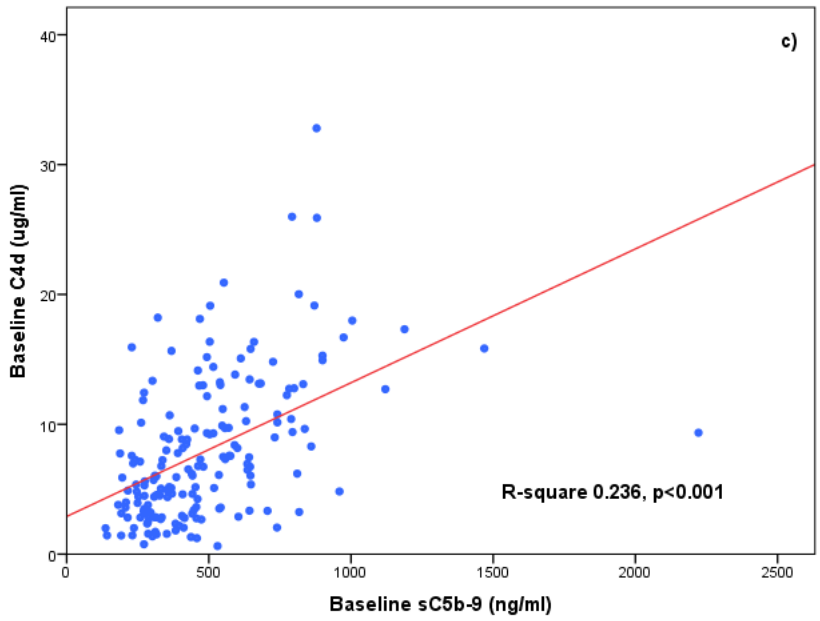



**Figure 4.** Prognostic accuracy of CAP for 730 days mortality

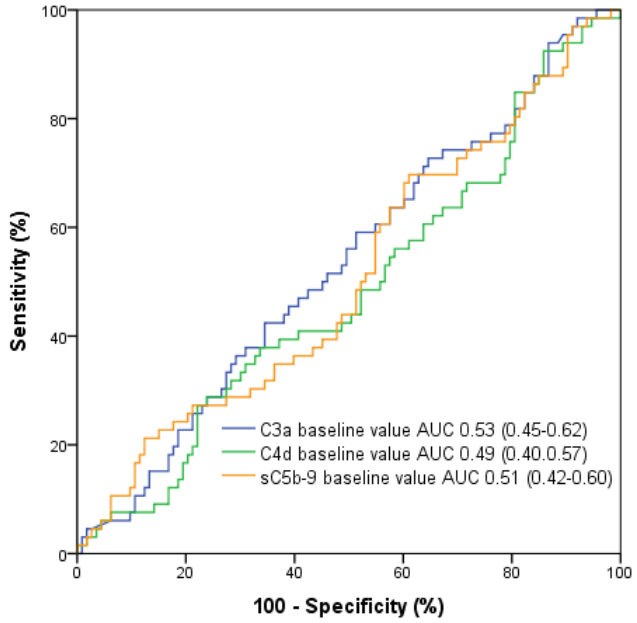
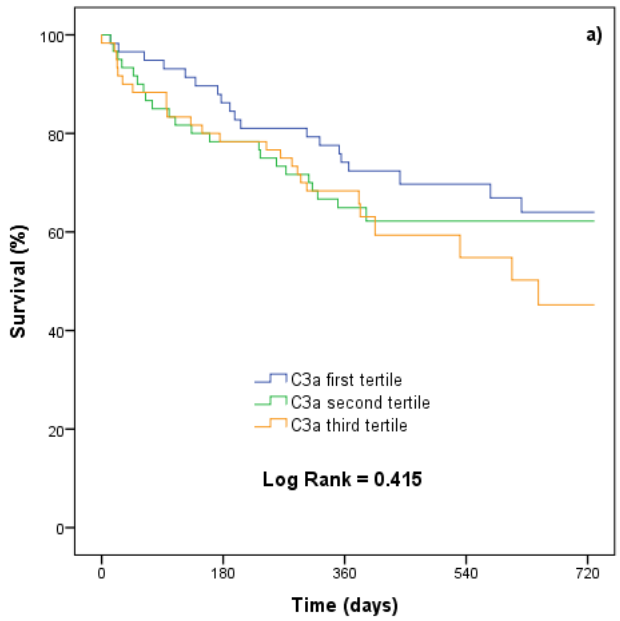
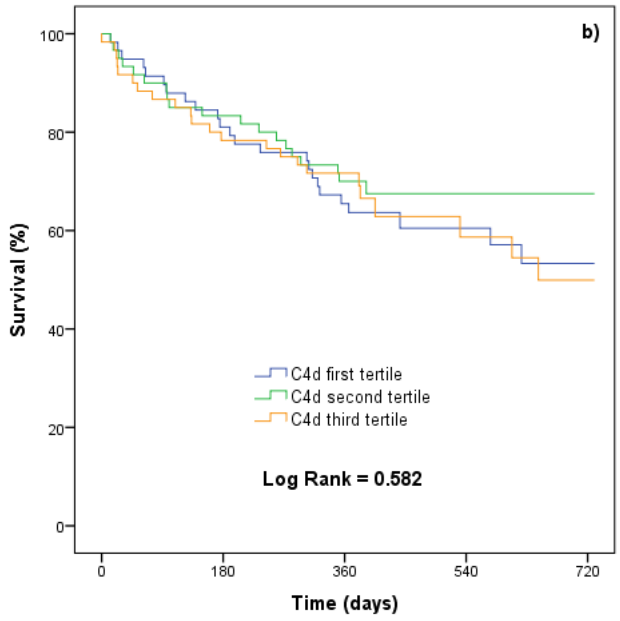
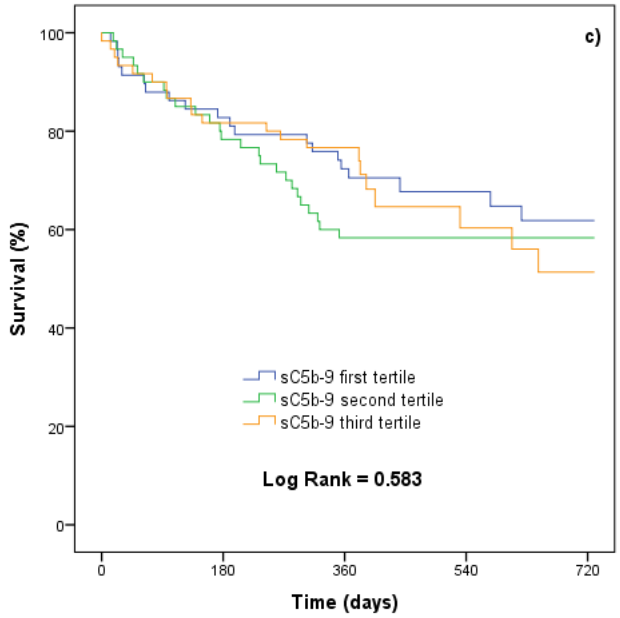



**Figure 5 (a), (b), (c)** Kaplan Meier for 730 days mortality for admission levels of C3a (a), C4d (b) and sC5b-9 (c)


a)
100
80
60
40
20
0
Survival (%)
C3a first tertile
C3a second tertile
C3a third tertile
Log Rank = 0.415
0
180
360
540
720
Time (days)


b)
100
80
60
40
20
0
Survival (%)
C4d first tertile
C4d second tertile
C4d third tertile
Log Rank = 0.582
0
180
360
540
720
Time (days)


c)
100
80
60
40
20
0
Survival (%)
sC5b-9 first tertile
sC5b-9 second tertile
sC5b-9 third tertile
Log Rank = 0.583
0
180
360
540
720
Time (days)