Complement C3c and C4c as predictors of death in heart failure ☆

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ABSTRACT

Background: Activation of the immune system is one of the several pathways suggested as involved in Heart Failure (HF). The complement system is a key component of innate immunity. We hypothesized that complement proteins C3 and C4 can be an important predictor of death in patients with this condition.
Methods: 380 patients admitted with acute HF were recruited. They were followed up during 6 months. Serum C3c and C4c proteins were measured and groups were created and compared according to the 25th percentile cut-off value. A multivariate Cox-regression model was used to establish the prognostic value of both markers with the endpoints of HF and all-cause death.
Results: Median patients' age was 78 years and 49% of the patients were men. No major differences were observed in clinical characteristics of the groups. Patients with lower values of C3c and C4c had significantly higher values of BNP. During the 6 month period of follow up, 63 patients died, and 49 patients were due to HF. C4c showed univariate prognostic value, but not multivariate value. The multivariate-adjusted Hazard Ratios for the 6 month HF and all-cause death in patients with C3c values below 110.0 mg/dL were, respectively, 2.32 (95% CI: 1.25–4.28) and 2.52 (95% CI: 1.41–4.49).
Conclusion: Lower C3c levels are independently associated with higher risk of death. Our results reinforce the role of innate immunity in HF pathophysiology.

1. Introduction

The knowledge on the pathophysiology of Heart Failure (HF) has evolved from a hemodynamic model to a more complex multisystem syndrome. Several pathways are pointed out as involved in the progression of this condition. Recently, the activation of the immune system has received considerable interest [1,2]. It is becoming increasingly apparent that inflammatory mediators play a crucial role in the development and perpetuation of HF. In fact, the auto-perpetuation observed in HF can be, in large part, due to an imbalance between inflammatory and anti-inflammatory mediators, in both ischemic and nonischemic HF [3,4]. These mediators are also known to be related to HF severity and associated with prognosis [2,5]. One of the hallmarks of HF pathogenesis is the remodeling process. There is now evidence that innate immunity has a determinant role in this process. In fact, after myocardial injury and damage of cardiac cells, innate immune system must be activated for a correct regeneration [6–8]. However, long-term chronic innate immune activation is detrimental as it leads to adverse left ventricular remodeling and HF worsening. This activation involves a variety of inflammatory molecules and pathways, such as proinflammatory cytokines, immune cells, autoantibodies formation and complement activation [9,10]. The complement system is a key component of the innate immune system and comprises a cascade of more than 30 proteins. The complement system has a dual role, as a receptor, for example, for host infection and as an effector protein that can efficiently attract inflammatory cells and also directly destroy cells by the membrane attack complex [11].

We hypothesized that in patients with an episode of acute HF, proteins from the complement system namely C3 and C4 can be involved in the pathophysiology of HF. We also hypothesized that besides their involvement, the magnitude of their activation can be related with HF outcome.

2. Methods

We conducted a prospective observational study between January 2009 and December 2010. During this period, patients admitted to the

Internal Medicine Department of a central Portuguese Hospital Center, with the primary diagnosis of HF, whether worsening or *de novo* HF, were eligible for study entry. Patients with acute coronary syndromes, with complaints attributable to causes other than HF, or with no echocardiographic structural or functional cardiac abnormalities were excluded.

Treatment decisions, timing of discharge, and discharge medication were at discretion of the attending physician and the physicians were aware of the ongoing registry.

An echocardiogram was performed within 72 h of admission to all eligible patients. Comprehensive echocardiographic assessment was performed using a multi-frequency matrix probe (Vivid6, GE Healthcare). The diagnosis of HF was made based on the European Society of Cardiology guidelines [12]. All HF etiologies were admitted. Patients with left ventricular systolic dysfunction (LVSD) and with HF with preserved ejection fraction were included in the registry. Normal systolic fraction was defined as a left ventricular ejection fraction (LVEF) above 50%. Treatment and time of discharge were decisions of the attending physician.

Fasting venous blood samples were collected from all patients between 7 and 8 am on discharge day. Clinical and demographic data, as well as other relevant information were collected by interview upon the collection of the blood sample. B-type natriuretic peptide (BNP) was measured by a chemiluminescent immunoassay in the Architect i2000 automated analyzer (Abbott). Creatinine and C-reactive protein (CRP) were measured in the automated clinical chemistry Olympus AU5400 analyzer (Beckman-Coulter). C1i, C3c and C4c complement proteins were measured in the Dimension Vista 1500 nephelometer (Siemens). Hemoglobin was evaluated in an automated blood counter Sysmex XE-5000 (Emilio de Azevedo Campos).

Comorbidities were also recorded for each patient. Coronary heart disease was defined as either history of myocardial infarction, history or electrocardiographic evidence of ischemia, or coronary angiography confirmation. Diabetes *mellitus* was defined as either a history of diabetes or the current prescription of either an oral hypoglycemic agent or insulin. Anemia was considered when hemoglobin level was below 13 g/dL in men and 12 g/dL in women. Arterial hypertension was defined as the presence of previous diagnosis and record of antihypertensive pharmacological treatment. Renal dysfunction was considered when creatinine levels exceeded 1.5 mg/dL. Estimated glomerular filtration rate (GFR) was calculated by the Cockcroft–Gault formula [13].

Patients were followed up during a 6 month period after their hospital discharge, by consulting hospital registries and/or by telephone contact. The endpoints were defined as HF death, including worsening congestion due to progressive pump failure and sudden cardiac death, and all-cause mortality.

All patients provided written informed consent to participate in the study. The study protocol conforms to the ethical guidelines of the declaration of Helsinki and was approved by the local ethics committee.

2.1. Statistical analysis

Continuous variables are presented as median (interquartile range) due to the skewed distribution. Normality of the variables was tested by the Shapiro–Wilk test. Categorical variables are presented as counts and proportions.

Patients were divided according to the cut-off value correspondent to the 25th percentile for C3c and C4c. The groups of patients created were compared. A Chi-Square test was used for the comparison of categorical variables. Mann–Whitney test was used for comparing continuous variables once their distribution was skewed.

Kaplan–Meier test was used for estimating the survival function of patients in the 6 month follow-up with the outcome of HF death.

Spearman's coefficient was calculated to evaluate the correlation between continuous variables.

A multivariate Cox regression analysis was used to assess the prognostic power of C3c and C4c. Variables with prognostic impact in an univariate approach or known to influence HF prognosis in all groups were also included in the multivariate model built.

The *p* value considered for statistical significance was 0.05 for a confidence interval of 95%.

Data was stored and analyzed using SPSS software (SPSS Inc, Chicago, Illinois, 20.0).

3. Results

We included 380 patients discharged after hospitalization due to an acute episode of HF. Patients had a median age of 78 years old (interquartile (IQ) range: 70–84) and half of them were men (48.7%). Ischemic HF etiology was considered in 36.3% of the patients, 56.6% had systolic HF and 19.3% were discharged with a New York Heart Association (NYHA) class of III or IV. Diabetes *mellitus* and anemia were comorbidities in 45.0% and 42.6% of the patients, respectively. Arterial hypertension affected 77.1% of the patients, while patients suffering from renal dysfunction were 22.9%. Almost half of the patients revealed to have alcohol habits and 36.3% were current or ex-smokers. Patients' characteristics, comorbidities and laboratory assessments are shown in Table 1. When divided into groups according to the cut-off value of the 25th percentile of C3c and C4c, no major differences were noted in clinical characteristics. However, patients with lower C3c values showed a significantly smaller percentage of history of arterial hypertension. These patients were also less likely to be discharged on statins. They presented higher values of BNP (median 1188.8 pg/mL vs 602.1 pg/mL) and lower values of C4c and C1i. Patients with lower C4c values had an identical trend in BNP, C3c and C1i values. At discharge, they were also less likely to be discharged on statins or on beta-blockers, when compared with the ones with higher values of C4c (Table 1).

Patients were followed up for 6 months after discharge. During this period among the 49 patients that died due to HF, 22 had low C3c values and 21 low C4 values. Estimates revealed a significantly higher risk of death, by HF or other cause, in patients with lower values of C3c and C4c upon discharge. Fig. 1 shows the survival curves estimator for the 6 month period according to discharge values of the 25th percentile for C3c (110.0 mg/dL) for HF death endpoint.

Table 2 presents the predictors of 6 month HF and all-cause death in our group of patients in a univariate approach. These predictors for HF death were ischemic etiology of HF, NYHA class, history of arterial hypertension, higher BNP and lower hemoglobin, GFR, C3c and C4c values upon discharge. Besides the strong predictor for HF, BNP, had a hazard ratio (HR) of 1.02 for HF death (95% CI: 1.01–1.03) and all-cause death (95% CI: 1.01–1.02) per each 100 mg/dL of increase. Besides BNP, the markers C3c and C4c were also associated with shorter survival with a HR of 0.98 (95% CI: 0.97–0.99) and 0.97 (95% CI: 0.95–0.99) for HF and a HR of 0.98 for both in all-cause death, per each mg/dL of decrease. Although C1i showed a significant difference between the groups created, it did not reveal a significant HR concerning both endpoints in study. The prescription of angiotensin converting enzyme inhibitor and beta-blockers showed to have a significant protective effect in both endpoints.

In the multivariate adjusted models, in continuous and quartile analysis, C3c association with 6 month endpoints was independent of the other prognostic predictors. The multivariate adjusted HR of 6 month HF and all-cause death, per mg/dL of C3c values was 0.99 (95% CI: 0.98–1.00). In patients with C3c values lower than 110.0 mg/dL, the multivariate adjusted HR was 2.32 (95% CI: 1.25–4.28) for HF death and 2.52 (95 %CI: 1.41–4.49) for all-cause death, comparing with patients with higher C3c values. For patients with C4c values lower than 25.15 mg/dL, the multivariate adjusted HR was not statistical significant, as well as in the continuous analysis. The multivariate adjusted models can be observed in Table 3.

Table 1
Patients' demographics, clinical and laboratory characteristics and discharge medication; comparison between C3c and C4c groups.

	All patients ($n = 380$)	Low C3c ($n = 96$)	High C3c ($n = 284$)	p value	Low C4c ($n = 95$)	High C4c ($n = 285$)	p Value
Clinical characteristics							
Age (years), median (IQR)	78 (70–84)	78 (70–84)	77 (70–84)	0.523	76 (69–83)	78 (71–84)	0.582
Male sex, n (%)	185 (48.7)	51 (53.0)	134 (47.0)	0.315	50 (52.6)	135 (47.4)	0.375
Ischemic etiology of HF, n (%)	138 (36.3)	29 (30.2)	109 (38.4)	0.236	31 (32.6)	107 (37.5)	0.512
Preserved LVSF, n (%)	162 (43.4)	45 (27.8)	117 (72.2)	0.370	34 (21.0)	128 (79.0)	0.101
NYHA class at discharge (III&IV vs I&II), n (%)	73 (19.3)	20 (27.4)	53 (72.6)	0.651	20 (27.4)	53 (72.6)	0.609
Comorbidities							
Diabetes mellitus, n (%)	171 (45.0)	36 (37.5)	135 (47.5)	0.088	44 (46.3)	127 (44.6)	0.767
Anemia history, n (%)	162 (42.6)	44 (45.8)	118 (41.5)	0.321	44 (46.3)	118 (41.4)	0.833
Chronic arterial hypertension, n (%)	293 (77.1)	64 (66.7)	229 (80.6)	0.010	70 (73.7)	223 (78.2)	0.293
Chronic renal dysfunction, n (%)	87 (22.9)	22 (22.9)	65 (22.9)	0.147	18 (18.9)	69 (24.2)	0.440
Smoking habits, n (%) current smoker/ex smoker	29 (7.6)/109 (28.7)	6 (6.3)/27 (28.1)	23 (8.1)/82 (28.9)	0.682	3 (3.2)/28 (29.5)	26 (9.1)/81 (28.4)	0.296
Alcohol habits, n (%)	171 (45.0)	45 (46.9)	126 (44.4)	0.730	43 (45.3)	128 (44.9)	0.931
Laboratory at discharge							
Hemoglobin (g/dL), median (IQR)	12.0 (10.8–13.5)	11.7 (10.2–13.0)	12.1 (10.8–13.6)	0.048	11.8 (10.4–13.2)	12.0 (10.8–13.5)	0.170
GFR (mL/min), median (IQR)	39.6 (29.2–52.0)	37.1 (25.8–50.6)	40.4 (29.9–52.7)	0.160	41.8 (31.9–51.3)	38.6 (28.1–52.4)	0.165
BNP (pg/mL), median (IQR)	725.8 (307.5–1369.9)	1188.8 (566.6–1683.2)	602.1 (262.3–1209.2)	<0.000	1080.0 (384.4–1735.8)	609.9 (271.1–1284.0)	0.001
CRP (mg/L), median (IQR)	12.2 (6.1–25.0)	12.1 (6.0–21.7)	12.2 (6.1–28.6)	0.388	12.5 (7.3–22.0)	11.9 (6.1–28.7)	0.427
C3c (mg/dL), median (IQR)	130.0 (110.0–149.8)	101.5 (87.1–106.0)	138.0 (126–155)	<0.000	110.0 (97.3–132.0)	135.0 (116.5–153.5)	0.000
C4c (mg/dL), median (IQR)	32.3 (25.2–41.1)	24.9 (18.9–29.3)	34.9 (28.8–43.5)	<0.000	19.3 (16.0–23.3)	35.9 (30.5–43.7)	0.000
C1i (mg/dL), median (IQR)	32.3 (19.0–36.1)	30.3 (26.8–33.2)	33.4 (30.0–37.2)	<0.000	30.7 (26.2–33.6)	33.0 (29.8–37.1)	0.000
Discharge medication							
ACEi, n (%)	259 (68.2)	66 (68.8)	193 (68.0)	0.784	65 (68.4)	194 (68.1)	0.984
ARA, n (%)	51 (13.4)	8 (8.3)	43 (15.1)	0.097	11 (11.6)	40 (14.0)	0.537
Spirolactone, n (%)	96 (25.3)	18 (18.8)	78 (27.5)	0.099	24 (25.3)	72 (25.3)	0.986
Beta-blocker, n (%)	303 (79.7)	73 (76.0)	230 (81.0)	0.384	68 (71.6)	235 (82.5)	0.019
Nitrates, n (%)	103 (27.1)	19 (19.8)	84 (29.6)	0.240	21 (22.1)	82 (28.8)	0.429
Statins, n (%)	247 (65.0)	47 (49.0)	200 (70.4)	<0.000	50 (52.6)	197 (69.1)	0.002
Diuretic, n (%)	357 (93.9)	89 (92.7)	268 (94.4)	0.713	92 (96.8)	265 (93.0)	0.306
HF death	49 (12.9)	22 (44.9)	27 (55.1)	0.001	21 (42.9)	28 (57.1)	0.002
All cause death	63 (16.6)	29 (46.0)	34 (54.0)	<0.000	24 (38.1)	39 (61.9)	0.009

IQR: interquartile range; HF: heart failure; LVSF: left ventricular systolic function; NYHA: New York Heart Association; GFR: estimated *glomerular filtration rate*; BNP: B-type natriuretic peptide; CRP: C-reactive protein; C1i: C1 inhibitor; ACEi: angiotensin converting enzyme inhibitor; ARA: angiotensin II receptor antagonist.

Table 4 presents the Spearman's correlations between the continuous variables in study. All complement proteins correlated with each other, with the stronger correlation between C3c and C4c. BNP had a negative correlation with C3c and C4c proteins. CRP only positively correlated with C3c.
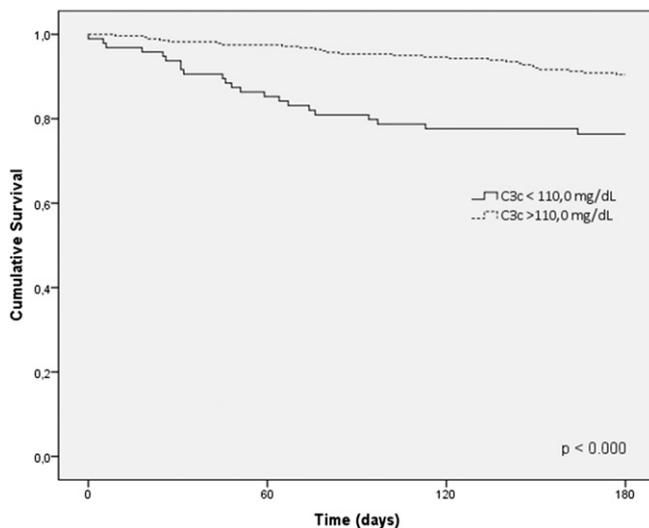
Fig. 1. Kaplan–Meier survival curves according to C3c cut-off values of the 25th percentile. Patients with lower values of C3c had higher 6 month risk of heart failure death after an acute heart failure episode.

4. Discussion

We report that low values of C3c and C4c upon discharge of an acute HF episode are associated with unfavorable outcome in HF patients. The multivariate adjusted risk of HF or all-cause death at 6 months for patients with C3c lower than 110.0 mg/dL was about 2.5 fold higher than for those with higher values at discharge. For C4c, although it showed prognostic value in a univariate approach, the same did not come through in a multivariate analysis.

The association between activation of the immune system and HF has been reported [14,15], with high levels of CRP as an indicative of severity of disease [16]. CRP mediates several protective processes, but may also have deleterious effects in HF, such as the up-regulation of tumor necrosis factor α (TNFα) and interleukin (IL)-6 which are a strong sign of this activation [3,4,17,18]. In fact, circulating levels of pro-inflammatory cytokines are enhanced in the failing myocardium, in both ischemic and nonischemic HF, and are long known to be related to disease severity and to predict poor survival [2,5]. Our data also corroborate this assumption. The levels of CRP, at discharge, had a median of 12.2 mg/L (IQR: 6.1–25.0) and showed a trend to predict HF death (p value = 0.063) and a univariate statistical significant prediction value for all-cause death (p value = 0.013). Thus, once these are markers of immune activation and knowing that after an initial heart insult, the increased production of proinflammatory cytokines may challenge the surrounding tissue through propagation of the inflammatory response and direct effects on the cardiac myocyte structure and function, it is reasonable to assume that the complement proteins may also contribute to the destructive action of these factors and to the pathogenetic sequelae of HF [14].

Table 2

Univariate Cox-regression between patients' characteristics and 6 month heart failure and all-cause death, after an acute heart failure episode.

	HF death		All-cause death	
	HR (95% CI)	p Value	HR (95% CI)	p Value
Clinical characteristics, all patients ($n = 380$)				
Age (per year)	1.02 (1.00–1.05)	0.111	1.03 (1.00–1.05)	0.041
Male sex	1.64 (0.92–2.93)	0.092	1.18 (0.72–1.94)	0.503
Ischemic etiology of HF	1.27 (1.05–1.54)	0.016	1.24 (1.03–1.50)	0.023
Preserved LVSF	1.56 (0.86–2.83)	0.146	1.01 (0.61–1.66)	0.983
NYHA class at discharge (III&IV vs I&II)	1.31 (1.08–1.60)	0.007	1.30 (1.09–1.55)	0.004
Comorbidities				
Diabetes mellitus	0.87 (0.65–1.16)	0.332	0.83 (0.64–1.07)	0.144
Anemia history	1.00 (0.76–1.32)	0.986	1.12 (0.95–1.32)	0.194
Chronic arterial hypertension	0.47 (0.26–0.85)	0.012	0.50 (0.30–0.86)	0.011
Chronic renal dysfunction	1.11 (0.90–1.37)	0.338	1.23 (1.09–1.40)	0.001
Smoking habits	1.15 (0.95–1.39)	0.166	1.09 (0.90–1.32)	0.364
Alcohol habits	0.92 (0.52–1.63)	0.781	0.80 (0.48–1.33)	0.394
Laboratory at discharge				
Hemoglobin (per g/dL)	0.85 (0.73–0.98)	0.030	0.83 (0.73–0.95)	0.007
GFR (per mL/min)	0.98 (0.97–1.00)	0.045	0.98 (0.97–1.00)	0.010
BNP (per 100 pg/mL)	1.02 (1.01–1.03)	<0.000	1.02 (1.01–1.02)	<0.000
CRP (per 10 mg/L)	1.08 (1.00–1.16)	0.063	1.09 (1.02–1.16)	0.013
C3c (per mg/dL)	0.98 (0.97–0.99)	<0.000	0.98 (0.98–0.99)	<0.000
C4c (per mg/dL)	0.97 (0.95–0.99)	0.005	0.98 (0.96–0.99)	0.009
C1i (per mg/dL)	0.97 (0.92–1.02)	0.251	0.99 (0.95–1.03)	0.625
Discharge medication				
ACEi	0.49 (0.28–0.86)	0.013	0.54 (0.33–0.89)	0.016
ARA	0.56 (0.20–1.56)	0.266	0.66 (0.28–1.53)	0.328
Spirolactone	0.74 (0.37–1.49)	0.397	0.82 (0.45–1.48)	0.509
Beta-blocker	0.39 (0.22–0.69)	0.001	0.49 (0.29–0.83)	0.008
Nitrates	0.82 (0.48–1.42)	0.483	0.86 (0.55–1.35)	0.505
Statins	0.70 (0.40–1.25)	0.226	0.72 (0.43–1.20)	0.207
Diuretic	0.85 (0.41–1.75)	0.657	0.89 (0.51–1.56)	0.691

HR: hazard ratio; IQR: interquartile range; HF: heart failure; LVSF: left ventricular systolic function; LVSD: left ventricular systolic dysfunction; NYHA: New York Heart Association; GFR: estimated *glomerular filtration rate*; BNP: B-type natriuretic peptide; CRP: C-reactive protein; C1i: C1 inhibitor; ACEi: angiotensin converting enzyme inhibitor; ARA: angiotensin II receptor antagonist.

Evidence also showed that extrahepatic tissues, such as the heart, produce complement proteins in ischemic and nonischemic conditions [19,20]. As low complement levels are normally an indicator of high tissue complement turnover [21,22], the low (peripheral) levels in patients in our study may represent an increased (local) activation of complement in the heart. This activation and consequent lower values can be associated with the poor outcome of our patients, also supported with the evidence that these patients had significant higher BNP values, representing patients at a higher risk of death due to HF [23]. In fact, by the Spearman correlation C3c and C4c values significantly correlate with BNP values in a negative way. In the heart, activated complement C3 was already shown to cause tachycardia, impairment of atrioventricular conduction, left ventricular contractile failure, coronary vasoconstriction, and histamine release after injection into isolated guinea pig hearts [24]. Thus, besides being a good marker for overall complement activation, C3 seems to be of pathophysiological relevance in the cardiovascular system.

The consumption of serum C3 and C4 reflects the activation of the classic and alternate pathways of the complement system [22]. CRP values besides representing a state of chronic immune activation may also contribute to the understanding of complement activation. CRP activates the classical pathway of complement when bound to appropriate ligands. CRP efficiently activates the early proteins of the classical pathway (C1, C2, C4 and to a lesser extent C3) but does not generate consumption of the components of the membrane attack complex (C5–C9) [25]. Surface-bound CRP decreases the alternative complement pathway C3-convertase and C5-convertase activities, inhibits the alternative amplification loop and reduces deposition of C3b and lysis by the lectin pathway [26]. In our patients the values of CRP did correlate positively with C3c values, but not with C4c. This positive relationship suggests that CRP can be related with the activation of C3c, by the

Table 3

Multivariate Cox-regression model for C3c and C4c on 6 month HF death and all-cause death, after an acute HF episode.

	HF death[a]		All-cause death[b]	
	HR (95% CI)	p Value	HR (95% CI)	p Value
C3c (per mg/dL)	0.99 (0.98–1.00)	0.030	0.99 (0.98–1.00)	0.027
C4c (per mg/dL)	0.98 (0.96–1.01)	0.149	0.98 (0.96–1.00)	0.097
C3c (1st Q vs others)	2.32 (1.25–4.28)	0.008	2.52 (1.41–4.49)	0.002
C4c (1st Q vs others)	1.84 (0.99–3.42)	0.055	1.66 (0.92–3.00)	0.091

HR: hazard ratio; HF: heart failure.

[a] Model adjusted for: ischemic etiology of heart failure; New York Heart Association class at discharge (III&IV vs I&II); chronic arterial hypertension; hemoglobin (per g/dL); estimated *glomerular filtration rate*; B-type natriuretic peptide (per 100 pg/mL); angiotensin converting enzyme inhibitor; beta-blocker medication.

[b] Model adjusted for: age (per year); ischemic etiology of heart failure; New York Heart Association class at discharge (III&IV vs I&II); chronic arterial hypertension; chronic renal dysfunction; hemoglobin (per g/dL); estimated *glomerular filtration rate*; B-type natriuretic peptide (per 100 pg/mL); C-reactive protein (per 10 mg/L); angiotensin converting enzyme inhibitor; beta-blocker medication.

Table 4

Spearman's correlations.

		C1i	C3c	C4c	CRP	BNP
C1i	Correlation coefficient	–	0.369[*]	0.295[*]	0.305[*]	−0.070
	p value		<0.000	<0.000	<0.000	0.174
C3c	Correlation coefficient	0.369[*]	–	0.515[*]	0.106[*]	−0.308[*]
	p value	<0.000		<0.000	0.040	<0.000
C4c	Correlation coefficient	0.295[*]	0.515[*]	–	0.057	−0.217[*]
	p value	<0.000	<0.000		0.267	<0.000

C1i: C1 inhibitor; CRP: C-reactive protein; BNP: B-type natriuretic peptide.

[*] Significant correlation.

alternative and lectin pathways. On the other hand, CRP correlated with C1i in a positive way. The main function of C1i is the inhibition of the classical pathway of complement to prevent spontaneous activation, with levels raising about 2 fold during inflammation [27,28]. In this study, the values of C3c and C4c positively correlated with C1i values. This correlation shows that the activation of the classical pathways due to a non-inactivation by C1i may also be present in HF.

To our knowledge, this study is the first one to strongly establish a significant role for complement as a predictor marker of HF death in an adjusted survival model. Previous studies have evaluated the presence and prognostic role of complement system in HF. A study with a small number of patients (36 participants) with HF registered complement activation in these patients [29]. In this same study, they have also shown an association of high SC5b9 levels with 6 month poor outcome, in an unadjusted model of survival. Another small study with a similar number of patients with HF (39 participants), also reported a systemic complement activation attenuated with intravenous immunoglobulin treatment [30]. In a larger study with 118 CHF patients, SC5b9 was also evaluated and correlated with CRP levels. Nevertheless, in these last two studies, the role as a predictor of death was not evaluated. Clearly, although these studies represent a necessary uncover of the subject potentiating other studies, the small number of participants and the absence of adjusted models are major weaknesses. A more recent study in 182 patients reported a significant association between complement C3a higher values and a combined endpoint of all-cause mortality or rehospitalization due to progression of HF [31]. In this study, the authors only focused in the analysis of the anaphylotoxin C3a, although also evaluating C3c and C4c levels. It is true that C3a is a complement activation product with powerful biological effects; nevertheless this product is rather unstable and degradable upon freezing. In our study, we measured C3c and C4c, which are more stable complement products [32]. In another recent study with 197 patients with stable HF, the authors reported that patients with higher C3c levels exhibited a trend to better survival but were unable to statistically prove that prediction [33]. The major problem in that report was the division of the patients according to the assay reference intervals, clearly not the proper way of studying the value of a biomarker, as was our intention in our cohort. In our study we divided the patients according to quartiles and in this way we could show an association between those that belonged to the first quartile, with the likelihood of dying due to complications of HF. Also, and differently from the two studies referred, as all patients were evaluated at discharge, we may assume with some level of certainty that they were in the same degree of immune activation.

5. Conclusion

Our results show that upon hospital discharge after an episode of acute HF, lower values of C3c are associated, BNP independently, with an approximately 2.5 higher risk of 6 month HF and all-cause death. Our results suggest that this marker can have a role in the identification of patients at increased risk of mortality.

Conflict of interests

The authors declare no conflict of interests, including specific financial interests and relationships relevant to the subject of the paper.

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