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Free light chains a novel biomarker of cardiovascular disease. A pilot study

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Abstract. – OBJECTIVE: Atherosclerosis and ischemic heart disease (IHD) are the major cause of morbidity and mortality but their inflammatory pathogenesis is still unclear. In this scenario, the role of serum free light chains (sFLC) has never been fully evaluated. The aim of the present study is to assess the clinical and pathogenetic role of sFLC in patients with IHD and to propose their use as a new biomarker for cardiovascular disease.

PATIENTS AND METHODS: We enrolled 117 patients, divided into 5 cohorts: 15 healthy controls, non-diabetic and without ischemic heart disease; 19 patients with type 2 diabetes (T2DM), without ischemic heart disease at recruitment; 39 patients with stable chronic angina; 27 patients with NSTEMI, 17 patients with acute STEMI. Serum sFLC and high-sensitive C-reactive protein (hs-CRP) were measured. Patients also underwent a transthoracic echocardiographic study.

RESULTS: sFLC were higher in patients with IHD and T2DM. However, we did not find statistically significant differences in sFLC concentration among subgroups. No correlation resulted between sFLC and hs-CRP levels. The median value of the sFLC κ/λ ratio in the population was 0.63, therefore stratifying it into two groups according to their levels. We found that an increase in left ventricular ejection fraction at 12 months was detected in 77% of patients with κ/λ ratio higher than 0.63 and 25% of patients with κ/λ ratio lower of 0.63 (p=0.016, OR=10.0 [1.8-55.6]).

CONCLUSIONS: Our study suggests that the sFLC, produced by the B-lymphocytes in the context of generalized immune activation, could play a pathogenetic role in acute coronary syndromes and that they could represent a novel risk biomarker of cardiovascular disease.

Key Words
FLC, Acute coronary syndromes, Diabetes mellitus.

Abbreviations

ACS: acute coronary syndrome; hs-CRP: high-sensitive C-reactive protein; HSP27: heat shock protein 27; IHD: ischemic heart disease; LVEF: left ventricular ejection fraction; NSTEMI: non-ST elevation acute myocardial infarction; STEMI: ST elevation acute myocardial infarction; sFLC: serum free light chains; T2DM: type 2 diabetes mellitus.

Introduction

Atherosclerosis and ischemic heart disease (IHD) are the main cause of morbidity and mortality in many countries; however, we are not yet able to fully explain the heterogeneity of pathophysiology and its clinical implications. In particular, there is a strong interest in innate and adaptive immune response, which is implicated in various phases of atherogenesis, contributing to early endothelial dysfunction and to progression and evolution of the lesion until the onset of acute coronary syndromes[1-3]. Until now the role of commonly used clinical indicators in emergency medicine was analyzed, such as age, sex, blood pressure, heart rate, blood glucose, NT-proBNP and other indicators in the diagnosis and classification of heart failure[4,5]; little attention has been addressed to serum free light chain (sFLC). The excess protein production without a reason or a specific function is rare in a biological system; therefore, sFLC

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should be considered bioactive molecules rather than a secondary product of the synthesis of immunoglobulins without any functional relevance[6]. sFLC are present in low concentrations in many biological liquids, such as serum, urine, and synovial fluid[7]. An increase in serum levels may be due to various clinical situations such as inflammatory diseases, renal failure, depression, and plasma cell dyscrasia[8], and are conventionally associated with monoclonal gammopathies. However, a polyclonal increase, both for kappa and lambda chains, may occur in autoimmune and other chronic inflammatory conditions[6].

In this context, sFLC, produced by the B-lymphocytes during generalized immune activation, may play a role in IHD and participate to the occurrence of acute coronary syndrome. Bellary et al[9] suggested their use for the stratification of cardiovascular risk in patients with or without type 2 diabetes mellitus (T2DM); Shantsila et al[10] proposed their use as a predictive marker of mortality in patients with acute heart failure and acute coronary syndromes[9-11]. Therefore, due to the easy and relatively low cost of assessment, sFLC might represent a novel biomarker for cardiovascular disease.

On the basis of these previous investigations, we assessed the clinical and pathogenetic role of sFLC in IHD patients and in subjects with T2DM, which represents a strong risk factor and leads to a worse outcome for IHD population. To explore the possible contribution to the pathogenesis of the disease, we also compared sFLC levels to high sensitive C-reactive protein (hs-CRP) concentration, a well-known marker of inflammatory and cardiovascular risk. We also studied twenty-nine patients at follow-up after one year, including echocardiographic assessment of left ventricle ejection fraction (LVEF) to assess the long-term role of sFLC.

Patients and Methods

Population Study

We enrolled 117 patients, divided into 5 cohorts, as follows: 15 healthy controls, non-diabetic and without ischemic heart disease; 19 affected by T2DM, without ischemic heart disease at recruitment; 39 patients with stable chronic angina (SA); 27 patients with non-ST elevation acute myocardial infarction (NSTEMI), 17 patients with ST-elevation acute myocardial infarction (STEMI). Patients with ischemic events (SA, NSTEMI, and STEMI) were recruited at the clinical presentation (during hospitalization), whilst diabetic patients and healthy controls were recruited during outbound controls.

All individuals gave their written informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The Ethics Committee of the Fondazione Policlinico Universitario "A. Gemelli" – Catholic University of Rome approved the study (prot. 8936/15).

Determination of sFLC and hs-CRP Concentration

sFLC and hs-CRP were assayed for each patient. Venous withdrawal of 6 mL of blood was performed during the outpatient visit for the diabetic patients and healthy controls, and during hospitalization, before any possible percutaneous coronary intervention, for patients with ischemic heart disease. Samples were processed within two hours, centrifuged at 2500 g for 10 minutes, aliquoted and stored at -80°C until analyzed. For the sFLC assay, samples were tested using the N-Latex FLC test method by the Siemens BN II (Siemens Healthcare Diagnostics Ltd, Erlangen, Germany) nephelometer, following the manufacturer's instructions. Specimens were thawed once, keeping them at room temperature and immediately analyzed. The normal range for FLC κ and λ is 6.7-22.4 and 8.3-27 mg/L, respectively. The κ/λ ratio and the sum of κ+λ were also calculated. Detection of hs-CRP levels was performed using a nephelometric method following the manufacturer's instructions (Siemens Healthcare Diagnostics Ltd, Erlangen, Germany). The reference cut-off to define a positive sample for high hs-CRP was set at 5 mg/L, based on the 95th percentile value of a reference healthy control population.

Finally, the cohorts of patients with stable chronic angina, NSTEMI, and STEMI, underwent transthoracic echocardiographic study within three days from the enrollment, which evaluated LVEF and cardiac remodeling; in order to explore the evolution of clinical and laboratory picture, 29 patients had a clinical control and an echocardiographic study after 12 months.

The difference between LVEF at 12th month and at hospitalization was calculated for each patient (ΔLVEF = LVEF at 12th months – LVEF at the hospitalization) so that a positive value indicates an increase of the LVEF in the observed span time.

Table I. Cardiovascular risk factors.

	Age (yr)	Hypertension (No.)	Smoke (No.)	Hypercholesterolemia (No.)	Obesity (No.)
Healthy controls (N°=15)	60. 5±10. 5	0	1	0	0
Diabetes mellitus (No.=19)	67. 6±10. 5	14	2	14	13
Stable angina (No.=39)	64. 9±10. 3	25	6	14	8
NSTEMI (No.=27)	65. 8±10. 1	15	3	9	2
STEMI (No.=17)	62. 3±10. 2	12	6	8	1

Statistical Analysis

Collected datasets were reported on Microsoft Excel™ worksheets (Microsoft, Redmond, WA, USA). Statistical analysis was performed using the Statistical Package for Social Science (SPSS; SPSS Inc., Chicago, IL, USA), release 15.0. All data were first analyzed for normality of distribution using the Kolmogorov-Smirnov test of normality. Continuous variables were expressed as mean ± SD, categorical variables displayed as frequencies, and the appropriate parametric (Student's *t*-test or ANOVA) or non-parametric test (Mann-Whitney U-test, Kruskal-Wallis ANOVA, χ^2-test) was used to assess the significance of the differences between subgroups. Correlations were calculated by means of the Pearson correlation coefficient. A *p*-value of less than 0.05 was considered statistically significant.

Results

Tables I and II summarize the clinical and therapeutic features of the population under study. No correlation between sFLC levels and IHD classical risk factors or therapeutic approach was reported in literature[8]; thus, these factors did not contribute to confound the data analysis.

Levels of sFLC and hs-CRP

Both κ and λ sFLC were higher at enrollment in patients with IHD and T2DM in respect to controls (data not shown). In Figure 1 the κ+λ sFLC values were shown. In particular, a total concentration (mean±SD) of 28±10, 47±16, 38±19, 53±29 and 34±18 mg/L were observed for healthy controls, T2DM, SA, NSTEMI, STEMI groups, respectively.

We did not find statistically significant differences in sFLC concentration between controls and individual groups of patients, but for NSTEMI subgroup (p=0.004). However, the percentage of subjects with total sFLC levels higher than a cut-off determined on the basis of the 95th percentile of healthy controls (45 mg/L), was 37% in T2DM, 31% in SA, 52% in NSTEMI, and 29% in STEMI (Figure 2), indicating an heterogeneous distribution of values among subgroups.

In contrast, hs-CRP levels were higher in SA, NSTEMI and STEMI compared to controls (p<0.001, p<0.001 and p=0.002, respectively, data not shown). Moreover, these subgroups showed 11%, 39% and 53% of hs-CRP values higher than a cut-off of 5 mg/L (95° percentile of healthy subjects) and 20%, 45% and 53% higher than a cut-off of 3 mg/L, respectively, whilst they were lower than both cut-offs in 100% of healthy controls and diabetic patients (Figure 3). No correla-

Table II. Medical therapy at the enrollment

	Beta-blockers (No.)	ACE-i or ARBs (No.)	Ca-antagonists (No.)	Diuretic (No.)	Aspirin (No.)	Statin (No.)
Healthy controls (No.=15)	0	0	0	0	0	0
Diabetes (No.=19)	5	14	6	7	8	10
Stable angina (No.=39)	21	21	7	12	24	19
NSTEMI (No.=27)	16	19	9	7	26	115
STEMI (No.=17)	14	13	4	7	17	14

ARBs = Angiotensin Receptor Blockers; ACE-I = Angiotensin Converting Enzyme-inhibitors.

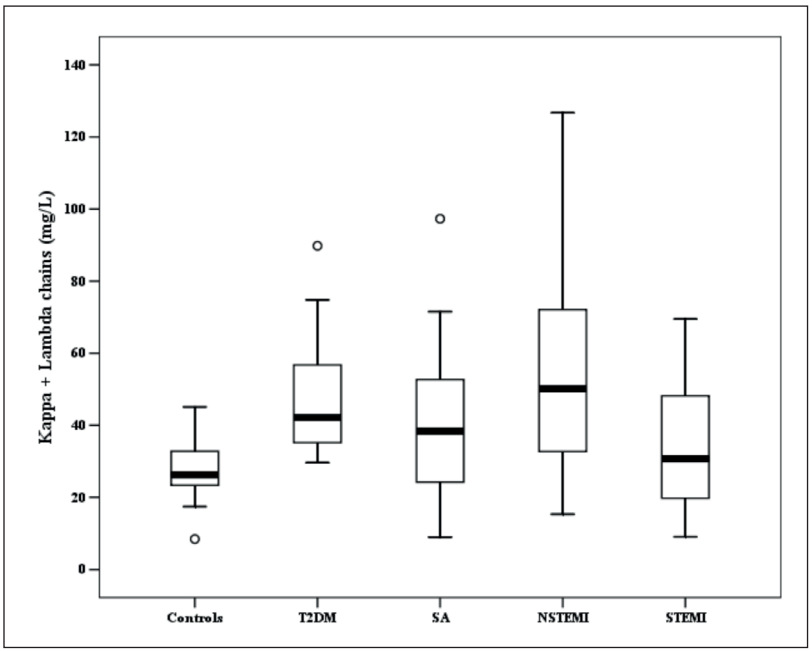

Figure 1. Box-plot diagram of κ+λ sFLC concentration in different study subgroups (T2DM: diabetes mellitus, SA: stable angina). ○ are cases with values between 1.5 and 3 times the Interquartile Range

tion was found between sFLC and hs-CRP levels.

Cardiac Remodeling Analysis

29 patients with stable chronic angina, NSTEMI and STEMI agreed to have a 12th-month follow-up (i.e., clinical visit and echocardiography). The variation of Left Ventricular Ejection Fraction (ΔLVEF) from recruitment was evaluated generating two subgroups: 1) subjects with an increase in LVEF higher than 5% and 2) subject showing a stable or decrease LVEF. A significant difference in λ (20±13 and 34±11 mg/L, p=0.006, respectively), κ/λ (0.70±0.130 and 49±0.15, p=0.001, respectively), and κ+λ (34±21 and 52±19 mg/L, p=0.025, respectively) sFLC concentrations at recruitment was detected between the two groups whilst no correlation was found with hs-CRP and κ sFLC. Finally, we stratified the population under study

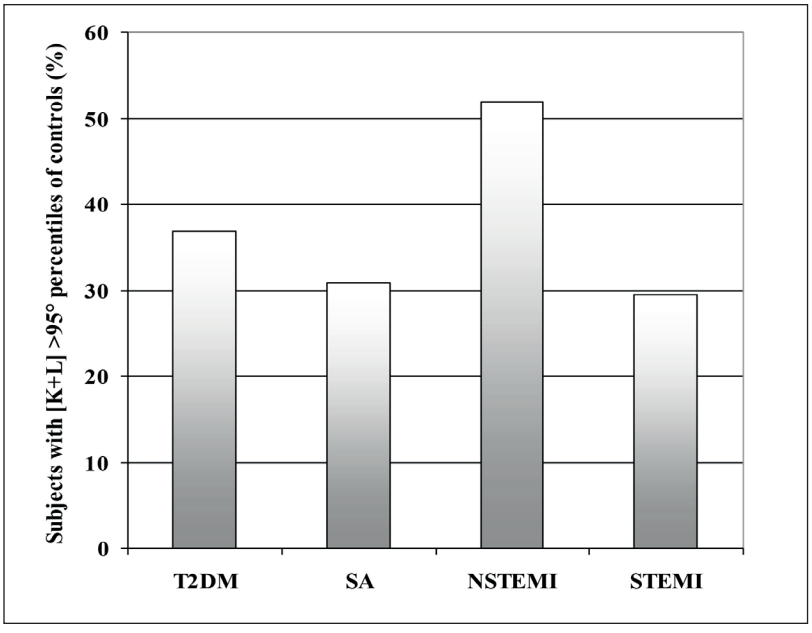

Figure 2. Frequency of subgroups subjects showing total sFLC levels higher than 95th percentile value of healthy controls. (T2DM: diabetes mellitus, SA: stable angina).

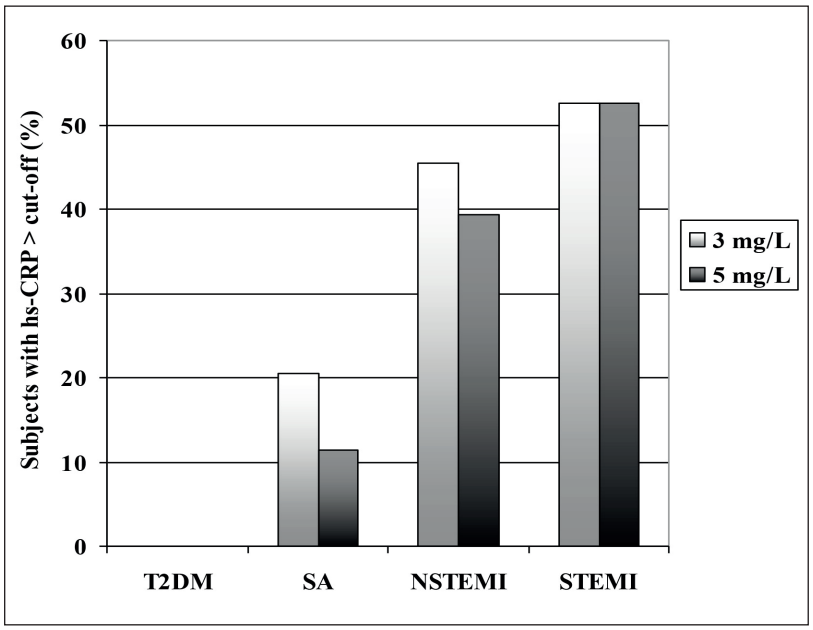

Figure 3. Frequency of subgroups subjects showing hs-CRP levels higher than 5 mg/L and 3 mg/L.

into two subgroups (equal/lower and higher than) according to κ/λ sFLC median levels (0.63), and the ΔLVEF was investigated. We found that an increase in LVEF at 12 months was detected in 77% of patients with κ/λ ratio higher than 0.63 and 25% of patients with κ/λ ratio equal/lower of 0.63 (p=0.016, Figure 4) with a calculated risk ratio of 10.0 [1.8-55.6].

Discussion

Serum FLC overproduction has been shown to be associated with an increased risk of mortality. Dispenzieri et al[12] followed up over 15,000 individuals aged 50 years or over who had undergone FLC analysis, and recorded mortality and cause of death. They demonstrated that a no

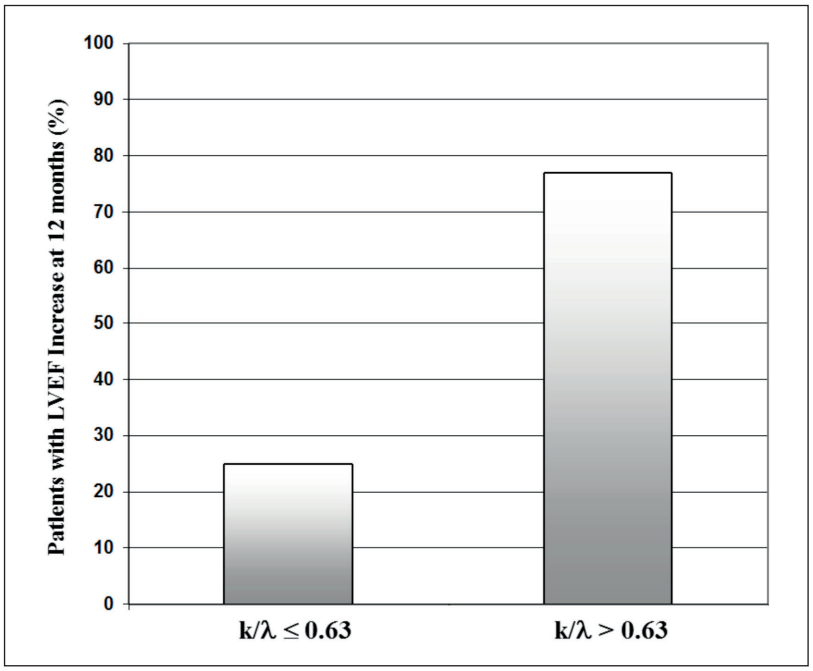

Figure 4. Patiens with LVEF improvement at 12 months from recruitment as a function of baseline κ/λ ratio.

clonal elevation of total sFLC was a significant, independent predictor of worse overall survival in the general population without plasma cell disorders[12].

The ability of sFLC to activate mast cells and become an active part of the pathogenic mechanisms of chronic inflammatory diseases has increased the interest in their clinical use, both as an attractive therapeutic target or as a biochemical marker of disease evolution or remission[6].

Burmeister et al[13] studied the relationship between sFLC and hs-CRP in order to assess the possible correlations between these markers in different chronic and acute diseases. The authors found that there was a weak correlation between polyclonal sFLC levels and hs-CRP, thus suggesting a different response kinetic profile of the two markers leading to complementary information on the inflammatory status[13].

Our study highlights the possible role of sFLC in the pathogenesis of cardiovascular diseases, as we found high values of these molecules in the serum of patients with ischemic heart disease (regardless of clinical presentation). Among patients with ischemic heart disease, sFLC values were particularly elevated in those with NSTEMI. However, the total sFLC levels spanned over a wide range, from low (9 mg/L) to extremely high concentration (>100 mg/L) independently from IHD clinical presentation, thus indicating an interindividual heterogeneity due to the associated pathology, timing of observation and disease stage. The non-specific connection of sFLC levels to IHD is also demonstrated by similar concentrations distribution observed among T2DM patients, a representative condition of non-specific inflammatory status. Moreover, hs-CRP was found increased only in IHD patients (11-53% of subjects with elevated hs-CRP concentrations, depending on clinical disease presentation), thus confirming a non-parallel behavior of the two markers in the population under study.

Finally, a high value of the κ/λ ratio at hospitalization has been shown to correlate with a 12-months LVEF improvement. Our work did not clarify the molecular mechanisms favoring the production of kappa chains over lambda. However, a possible explanation could be related, as suggested by numerous studies[14,15], to the protective role of the natural antibodies against atherosclerosis. The production of immunoglobulins after exposure of atheromatous plaque specific antigens could lead to an oligoclonal response with the increased serum concentration of a specific light chain category (κ or λ), resulting in an increase (or a decrease) of their ratio. Whether and which specific antibodies and sFLC are involved in an atheroprotective effect remains to be clarified, representing an interesting field for future research.

Conclusions

The fundamental role of innate immunity (with particular reference to chronic inflammation) in cardiovascular disease is well established, and the involvement of monocytes/macrophages in atherogenesis and tissue remodeling after insults is an example. On the other hand, only limited data exist between sFLC and cardiovascular disease, notwithstanding the amount of data on the role of adaptive immunity in IHD[16]. We showed that the sFLC, produced by the B-lymphocytes in the context of generalized immune activation, could play a role in this scenario and could be a novel risk biomarker of cardiovascular disease. Moreover, our results point out a possible correlation between a high κ/λ ratio and LVEF improvement.

Limitations of this work are the modest sample size and the lack of a consistent follow-up, this implies the need for extensive case studies to validate the results obtained. However, our results suggest a role of sFLC as a biomarker of IHD and in the pathogenesis of atherosclerosis, probably larger than so far considered.

Conflict of Interests

The authors have no conflicts of interest to disclose.

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