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Original Article

Inflammation is associated with the presence and severity of chronic coronary syndrome through soluble CD40 ligand

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Abstract: Introduction: Inflammation contributes to the initiation and progression of atherosclerosis, although the underlying inflammatory pathways are not entirely known. Specifically, the role of the proinflammatory soluble CD40 ligand (sCD40L) on the expression of chronic coronary syndrome (CCS) is not completely understood. We evaluated whether sCD40L expression is associated with the presence of CCS and with the clinical and anatomical severity of CCS. Methods: We prospectively recruited 94 participants, assigned to two groups matched by age and sex, without coronary artery disease (n=26) and with CCS (n=68). Clinical, laboratory and anatomical data were prospectively collected, and serum levels of sCD40L were measured. Results: In patients with CCS, classic cardiovascular risk factors were more prevalent, and the sCD40L levels, leukocyte and neutrophil counts, and neutrophil/lymphocyte ratio, but not the C-reactive protein levels, were significantly higher than those in controls. sCD40L was independently associated with the presence of obstructive coronary artery disease in multivariate analysis. Regarding CCS severity, sCD40L levels showed a significant stepwise increase with increasing angina severity (ANOVA P=0.001). In addition, sCD40L was independently associated with the anatomical severity of coronary artery disease, as assessed by the Gensini score. Among patients with CCS, those with previous coronary artery bypass grafting (n=23) had lower sCD40L levels than patients waiting for revascularization (n=45) [4.3 (2.1) ng/mL vs. 6.8 (3.5) ng/mL, P=0.001]. Conclusions: The expression of the proinflammatory sCD40L was associated with the presence of CCS and reflected the clinical and anatomical severity of CCS. In addition, we describe for the first time the association between prior CABG and reduced sCD40L levels in patients with CCS.

Keywords: Chronic coronary syndrome, coronary artery disease, inflammation, soluble CD40 ligand

Introduction

Atherosclerosis is recognized as an inflammatory disease, although the underlying inflammatory pathways associated with atherosclerotic disease initiation and progression are not completely understood [1-3]. Identifying major inflammatory mediators that participate in atherosclerosis regulation may contribute to not only a better understanding of pathophysiology but also clinical care, since they may potentially be used as diagnostic biomarkers and therapeutic targets, such as in the Canakinumab Antiinflammatory Thrombosis Outcome Study [1-4].

The soluble form of CD40 ligand (sCD40L) is a mediator of vascular inflammation that interacts with the CD40 receptor expressed on different cells involved in atherogenesis, including macrophages, endothelial cells and T-cells [5, 6]. Furthermore, we have previously observed that sCD40L expression is associated with vascular function [7, 8]. Altogether, the role of

sCD40L in vascular inflammation and function supports sCD40L as a mediator of atherosclerosis expression [5-8]. sCD40L has been extensively studied mechanistically and as a potential diagnostic and prognostic marker in patients with acute coronary syndrome [6-10]. Less is known about the role of sCD40L in chronic coronary syndromes (CCSs). CCS, a recently introduced concept that encompasses stable coronary artery disease, has a pathophysiology that is distinct from that of acute coronary syndrome since inflammation mainly regulates the initiation and progression of stable lesions in CCS; therefore, the study of inflammation specifically in CCS deserves special attention [1, 2, 11]. Some studies reported higher levels of sCD40L in patients with CCS than in controls [12-14], while other studies did not [15-20]. However, most of these studies have limitations regarding the sample size, method used for the exclusion of coronary artery disease, and lack of proper adjustment for confounding variables [12-20]. More importantly, there are few data on the relationship between sCD40L levels and the clinical or anatomical severity of CCS [20].

We aimed to evaluate whether the serum levels of sCD40L are associated with 1) the presence of CCS, and 2) the clinical and anatomical severity of CCS. We hypothesize that sCD40L levels are increased in CCS with obstructive coronary artery disease and that higher sCD40L levels are associated with increased severity of CCS.

Materials and methods

The study protocol was approved by the ethics committees of the involved institutions (Centro Hospitalar Universitário de Lisboa Central, Nr. 245/2015, and NOVA Medical School/Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Nr. 000176). The investigation conformed to the principles outlined in the Declaration of Helsinki. All participants signed informed consent forms.

Selection of participants

We prospectively recruited 94 participants followed in our center, which were assigned to two groups: 26 individuals without coronary artery disease (controls) and 68 patients with CCS.

Inclusion criteria: 1) Controls were recruited from the outpatient clinic and were eligible if they presented no effort angina; no evidence of coronary artery disease on coronary computed tomography angiography, including calcium score =0 and no soft plaques; and no positive myocardial stress test (the latter was not mandated to be assessed per protocol); and 2) Patients with CCS were recruited from those referred for elective invasive coronary angiography and were eligible in case of documented obstructive coronary artery disease, including luminal stenosis of at least 50% for the left main artery or at least 70% for other epicardial vessels.

Exclusion criteria: Patients with acute atherosclerotic events or coronary artery bypass grafting (CABG) within 12 months; those with any previous percutaneous coronary intervention, heart failure, hemodynamically significant valvular heart disease, hematological disorders, active infection, history of malignancy, chronic kidney disease (stage 4 or 5), or severe hepatic dysfunction; those under 18 years of age; or those unable or unwilling to consent to study participation were excluded. If performed at least 12 months before inclusion, CABG was not an exclusion criterion since the presence and extent of coronary artery lesions, which were the focus of this study, are not affected by the surgical placement of coronary artery bypass grafts [21].

Data collection

Clinical, laboratory and anatomical data were collected prospectively after patient inclusion. A standardized record of clinical and demographic characteristics (including cardiovascular risk factors, ongoing medication, clinical symptoms, and physical data), hematological and biochemical parameters, echocardiographic data, and angiography results was obtained from each participant. For the assessment of the anatomical severity of coronary artery disease, we assessed, for each patient, the number of obstructive lesions (luminal stenosis of at least 50% for the left main artery or at least 70% for other epicardial vessels); the number of vessels with obstructive lesions (the left main artery, left anterior descending artery, circumflex artery and right coronary artery were considered separately, with total score

ranging from 0 to 4); and the Gensini score, which was calculated as previously described [22].

Blood sampling and sCD40L measurements

Peripheral blood was collected early in the morning under fasting conditions. Serum was separated by centrifugation (500 g for 10 min) within 15 min of sampling. Aliquots were stored at -80°C; samples were thawed only once. Concentrations of sCD40L were measured in serum by an enzyme-linked immunosorbent assay commercial kit (R&D Systems). Each sample was measured in duplicate. The intra-assay variation among the duplicates for all samples was less than 10%.

Sample size

A sample size of 20 controls and 60 patients with CCS simultaneously provided: 1) 80% power to detect a 20% difference in sCD40L levels between controls and patients (for the first objective), with a standard deviation (SD) of sCD40L levels of 35% [12-14], at a 5% significance level; 2) a number of patients with CCS enough to carry out a comprehensive analysis on the association between sCD40L levels and CCS severity (for the second objective), including a potential multivariate linear regression analysis [23, 24]. The recruitment was carried out in a control:patient ratio of 1:3, matching by age and sex. Accounting for potential dropouts, additional participants were recruited. There were no dropouts and a total of 26 controls and 68 patients were included.

Statistical analysis

Discrete data are presented as frequencies (percentages), and continuous variables are presented as the mean (SD) or the median (interquartile range, IQR). Continuous variables were analyzed using Student's t-test or the Mann-Whitney test when normality was not verified (Shapiro-Wilk test); analysis of variance (ANOVA) was used for comparisons between multiple patient groups, and Bonferroni post hoc correction was used for multiple comparisons. Categorical variables were analyzed using the chi-squared or Fisher's exact tests. Pearson's correlation was used to test correlations between continuous variables. Multivariate logistic regression analysis was applied to identify which parameters were independently associated with the presence of obstructive coronary artery disease and (post hoc analysis) with previous CABG. Multivariate linear regression analysis was used to identify the independent predictors of the anatomical severity of coronary artery disease, as assessed by the Gensini score. Outliers were excluded, as appropriate [25]. The level of significance considered was α=0.05. Analyses were conducted with SPSS software, version 26 (IBM).

Results

Clinical and laboratory characteristics of participants

The characteristics of the 26 controls and 68 patients with CCS are presented in **Table 1**. In patients with CCS, hypertension, dyslipidemia, diabetes, a smoking history (current or past), and the use of antiplatelet and statin therapy were significantly more prevalent. In the CCS group, leukocyte count, neutrophil count, neutrophil/lymphocyte ratio, the percentage of glycosylated hemoglobin, creatinine levels, and sCD40L levels were significantly higher, and high-density lipoprotein (HDL) cholesterol levels were significantly lower, than in the control group. Of note, C-reactive protein levels did not differ between groups.

Association of sCD40L with clinical and laboratory data

sCD40L showed a weak positive correlation with leukocyte count, neutrophil count, lymphocyte count, and platelet count and a weak negative correlation with HDL-cholesterol levels (**Table 2**). The sCD40L levels did not differ according to age, sex or other cardiovascular risk factors and showed no correlation with other laboratory parameters, including C-reactive protein levels (**Table 2**).

sCD40L and the presence of obstructive coronary artery disease

Considering all clinical and laboratory data, sCD40L was an independent predictor of obstructive coronary artery disease in the multivariate logistic regression analysis, in addition to hypertension and HDL-cholesterol levels (**Table 3**).

Table 1. Clinical, laboratory and anatomical data of controls and patients with chronic coronary syndrome

	Controls (n=26)	Patients with CCS (n=68)	*p*-value
Clinical data			
Age, years	61.3 (9.4)	64.8 (9.3)	0.099
Male, n (%)	23 (88.5)	61 (89.7)	1.000
Hypertension, n (%)	14 (53.8)	64 (94.1)	<0.001
Dyslipidemia, n (%)	18 (69.2)	65 (95.6)	<0.001
Diabetes mellitus, n (%)	3 (11.5)	29 (42.6)	0.007
Smoking history (current or past), n (%)	6 (23.1)	37 (54.4)	0.006
Previous coronary artery bypass grafting, n (%)	0 (0.0)	23 (33.8)	<0.001
Left ventricular ejection fraction > 50%, n (%)	26 (100.0)	68 (100.0)	-
Antiplatelet agent, n (%)	6 (23.1)	66 (97.1)	<0.001
Statin therapy, n (%)	13 (50.0)	61 (89.7)	<0.001
Laboratory data			
Hemoglobin, g/dL	13.9 (1.3)	13.6 (1.6)	0.380
Leukocyte count, 10^9/L	6.4 (1.7)	7.6 (1.8)	0.012
Neutrophil count, 10^9/L	3.5 (1.5)	4.4 (1.5)	0.010
Lymphocyte count, 10^9/L	2.0 (0.5)	2.1 (0.8)	0.722
Neutrophil/lymphocyte ratio	1.9 (0.7)	2.4 (1.1)	0.029
Platelet count, 10^9/L	230.1 (58.8)	222.6 (51.1)	0.546
Fasting glycaemia, mg/dL	95.3 (35.1)	112.7 (52.5)	0.124
Percentage of glycosylated hemoglobin	5.8 (1.2)	6.4 (1.5)	0.014
Creatinine, mg/dL	0.8 (0.2)	1.0 (0.3)	0.001
Total cholesterol, mg/dL	186.0 (50.7)	166.4 (45.9)	0.082
LDL-cholesterol, mg/dL	111.3 (44.2)	100.9 (37.7)	0.261
HDL-cholesterol, mg/dL	51.5 (13.1)	37.6 (9.1)	<0.001
Triglycerides, mg/dL	133.6 (50.9)	149.0 (55.8)	0.234
C-reactive protein, mg/L	4.1 (2.0)	3.7 (1.7)	0.334
sCD40L, ng/mL	4.0 (1.5)	5.9 (3.3)	0.018
Anatomical data			
Nr. of vessels with obstructive disease	0 (0.0)	2.9 (0.8)	<0.001
Nr. of obstructive lesions	0 (0.0)	4.0 (1.4)	<0.001
Gensini score	0 (0.0)	72.9 (42.5)	<0.001

Data are expressed as mean (SD), except if otherwise specified. CCS - chronic coronary syndrome; HDL - high-density lipoprotein; LDL - low-density lipoprotein; Nr. - number; sCD40L - soluble CD40 ligand.

sCD40L and the severity of obstructive coronary artery disease

Clinically, the sCD40L levels showed a stepwise increase with increasing severity of angina among patients with obstructive coronary artery disease (ANOVA P=0.001) (**Figure 1**). Anatomically, there was a weak positive correlation between sCD40L levels and the number of coronary artery vessels with obstructive disease (r=0.285, P=0.006), the number of obstructive coronary artery lesions (r=0.238, P=0.022), and the Gensini score (r=0.279, P=0.007). The levels of sCD40L were higher in higher categories of the Gensini score (ANOVA P=0.026) (**Figure 2**).

The univariate analysis assessing parameters associated with the Gensini score (as a continuous variable) is presented in **Table 4**. In the multivariate linear regression analysis, sCD40L was an independent predictor of coronary artery disease severity, as assessed by the Gensini score, in addition to neutrophil/lym-

Table 2. Association of soluble CD40 ligand with clinical and laboratory parameters

		sCD40L, ng/mL[a]	*p*-value
Clinical parameters			
Age, years[b]		r=-0.085	0.419
Sex[c]	Male	5.5 (3.2)	0.876
	Female	5.4 (3.0)	
Hypertension[c]	No	4.9 (2.4)	0.555
	Yes	5.5 (3.1)	
Dyslipidemia[c]	No	5.3 (2.4)	0.953
	Yes	5.4 (3.1)	
Diabetes mellitus[c]	No	5.5 (3.0)	0.555
	Yes	5.2 (3.1)	
Smoking history[c]	No	5.1 (2.7)	0.363
	Yes	5.9 (3.3)	
Laboratory parameters			
Hemoglobin, g/dL[b]		r=0.123	0.243
Leukocyte count, 10^9/L[b]		r=0.301	0.004
Neutrophil count, 10^9/L[b]		r=0.219	0.037
Lymphocyte count, 10^9/L[b]		r=0.292	0.005
Neutrophil/lymphocyte ratio[b]		r=-0.041	0.704
Platelet count, 10^9/L[b]		r=0.223	0.035
Fasting glycaemia, mg/dL[b]		r=0.114	0.284
Percentage of glycosylated hemoglobin[b]		r=0.136	0.217
Creatinine, mg/dL[b]		r=0.118	0.258
Total cholesterol, mg/dL[b]		r=-0.017	0.874
LDL-cholesterol, mg/dL[b]		r=0.014	0.897
HDL-cholesterol, mg/dL[b]		r=-0.222	0.036
Triglycerides, mg/dL[b]		r=0.039	0.717
C-reactive protein, mg/L[b]		r=0.095	0.388

[a]Soluble CD40 ligand levels are expressed as mean (SD); [b]Correlations between soluble CD40 ligand levels and continuous variables were tested and the correlation coefficient (r) is presented for each; [c]soluble CD40 ligand levels were compared between groups for categorical variables. HDL - high-density lipoprotein; LDL - low-density lipoprotein; sCD40L - soluble CD40 ligand.

phocyte ratio, creatinine levels, and HDL-cholesterol levels (**Table 5**).

sCD40L and previous coronary revascularization

Among the 68 patients with CCS, 23 had previously undergone CABG (median 36 months, IQR 14 months to 10 years, before recruitment), and 45 had not been previously revascularized. Patients with prior CABG showed lower levels of sCD40L [4.3 (2.1) ng/mL vs. 6.8 (3.5) ng/mL, P=0.001]. Leukocyte, neutrophil, and platelet counts were also significantly lower in patients with prior CABG (**Table 6**). There were no other differences between groups, including the extent of native coronary artery disease (**Table 6**). In the multivariate logistic regression analysis, sCD40L was the only parameter independently associated with previous surgical revascularization (β 0.736, 95% confidence interval 0.597 to 0.908, P=004). There was no correlation between time elapsed from CABG and sCD40L levels (r=-0.263, P=0.225). When patients with prior CABG were classified according to the median time elapsed from CABG (36 months), there were no differences regarding the severity of coronary artery disease (**Table 7**) or sCD40L levels [5.0 (2.3) ng/mL vs. 3.6 (1.6) ng/mL, if time elapsed from CABG was inferior to or at least 36 months, respectively, p=0.098].

Of note, a post hoc multivariate analysis on predictors of obstructive coronary artery disease, excluding patients with CCS and prior CABG (**Table 8**), yielded similar results to the multivariate analysis including all patients with CCS (**Table 3**).

Discussion

In this prospective case-control study, three findings stood out: sCD40L levels were higher in patients with CCS; sCD40L levels were associated with the clinical and anatomical severity of coronary artery disease; and prior CABG was associated with reduced sCD40L levels among patients with CCS.

Regarding the increased levels of sCD40L in patients with CCS compared to controls, this association was independent from other clinical variables, such as classic cardiovascular risk factors, and from laboratory measures, such as those reflecting inflammatory activation, including leukocyte, neutrophil and plate-

Table 3. Predictors of obstructive stable coronary artery disease by multivariate logistic regression analysis

Predictors	β	95% CI	*p*-value
Hypertension	0.055	0.010 to 0.305	0.001
HDL-cholesterol, mg/dL	0.885	0.825 to 0.949	0.001
sCD40L, ng/mL	1.329	1.005 to 1.757	0.046

95% CI - 95% confidence interval; HDL - high-density lipoprotein; sCD40L - soluble CD40 ligand.

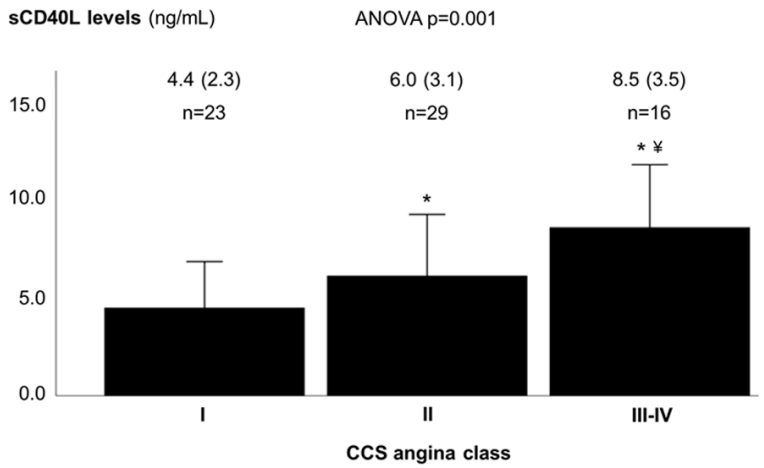

Figure 1. Soluble CD40 ligand levels according to angina class, among patients with obstructive coronary artery disease. Soluble CD40 ligand values are expressed as ng/mL, mean (SD). CCS - Canadian Cardiovascular Society (angina class); sCD40L - soluble CD40 ligand. * *p*-value <0.05 vs. angina class I; ¥ *p*-value <0.05 vs. angina class II.

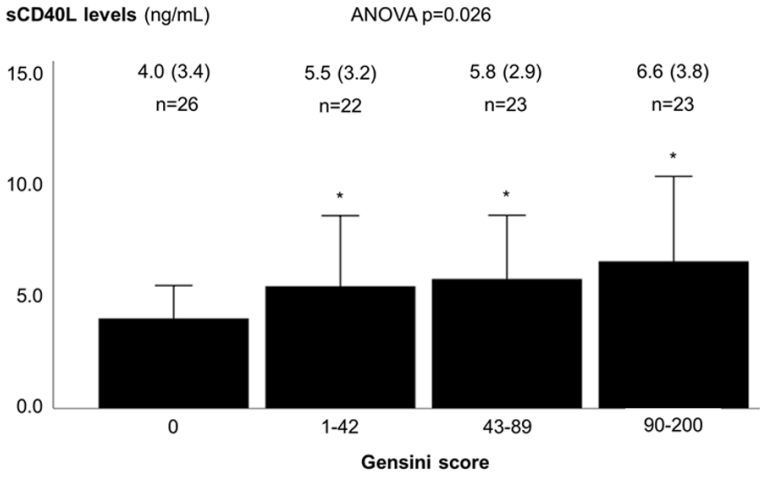

Figure 2. Soluble CD40 ligand levels according to the Gensini score. The first group corresponds to controls (all of which had a Gensini score of zero), and the remaining participants were equally divided into three groups according to the Gensini score. Soluble CD40 ligand values are expressed as ng/mL, mean (SD). sCD40L - soluble CD40 ligand. * *p*-value <0.05 vs. Gensini score of zero.

let counts and C-reactive protein levels. Some previous studies also reported higher levels of sCD40L in the presence of stable coronary artery disease [12-14], while others did not [15-20]. However, some of these studies have limitations regarding sample size [14, 16-19]. On the other hand, in previous studies, the exclusion of coronary artery disease in controls was based on invasive coronary angiography or on clinical assessment without an evaluation of coronary anatomy [12-20]. We used coronary computed tomography angiography to exclude coronary artery atherosclerosis in controls, which may have a higher negative predictive power than invasive coronary angiography [26]; indeed, invasive coronary angiography visualizes only luminal narrowing and may be insensitive to coronary artery lesions with positive remodeling and no compromise of luminal dimensions, which are conversely detectable by coronary computed tomography angiography [26]. In addition, most studies that tested sCD40L as a potential predictor of the presence of obstructive coronary artery disease lack proper adjustment for confounding variables [12, 14, 16-20]. We conducted a comprehensive multivariate analysis, which confirmed the independent association between sCD40L and coronary artery disease status.

Second, we observed that sCD40L was consistently and independently associated with the severity of coronary artery disease. There are few data available on this subject, and our results provide further insights into the pathophysiology of CCS presentation and severity. Previous studies reported no association between sCD40L levels and the anatomical severity of coronary artery disease, assessed by an angiographic score [12, 27] or by coro-

Table 4. Parameters associated with the Gensini score in univariate analysis

		Gensini score[a]	*p*-value
Clinical parameters			
Age, years[b]		r=0.201	0.053
Sex[c]	Male	53.6 (49.9)	0.877
	Female	45.3 (39.4)	
Hypertension[c]	No	25.9 (53.4)	0.002
	Yes	58.2 (46.2)	
Dyslipidemia[c]	No	12.4 (32.2)	0.001
	Yes	58.1 (48.2)	
Diabetes mellitus[c]	No	49.1 (51.4)	0.170
	Yes	59.7 (43.1)	
Smoking history[c]	No	54.5 (53.3)	0.105
	Yes	48.4 (36.2)	
Laboratory parameters			
Hemoglobin, g/dL[b]		r=0.066	0.530
Leukocyte count, 10^9/L[b]		r=0.294	0.005
Neutrophil count, 10^9/L[b]		r=0.265	0.011
Lymphocyte count, 10^9/L[b]		r=0.000	0.999
Neutrophil/lymphocyte ratio[b]		r=0.309	0.003
Platelet count, 10^9/L[b]		r=-0.048	0.650
Fasting glycaemia, mg/dL[b]		r=0.031	0.771
Percentage of glycosylated hemoglobin[b]		r=0.044	0.689
Creatinine, mg/dL[b]		r=0.322	0.002
Total cholesterol, mg/dL[b]		r=-0.149	0.156
LDL-cholesterol, mg/dL[b]		r=-0.064	0.543
HDL-cholesterol, mg/dL[b]		r=-0.450	<0.001
Triglycerides, mg/dL[b]		r=0.064	0.545
C-reactive protein, mg/L[b]		r=0.066	0.549
sCD40L, ng/mL[b]		r=0.279	0.007

[a]The Gensini score is expressed as mean (SD); [b]Correlations between the Gensini score and continuous variables were tested and the correlation coefficient (r) is presented for each; [c]The Gensini score was compared between groups for categorical variables. HDL - high-density lipoprotein; LDL - low-density lipoprotein; sCD40L - soluble CD40 ligand.

Table 5. Predictors of the Gensini score by multivariate linear regression analysis

Predictors	β	95% CI	*p*-value
Neutrophil/lymphocyte ratio	12.821	4.017 to 21.625	0.005
Creatinine, mg/dL	33.284	4.393 to 62.176	0.024
HDL-cholesterol, mg/dL	-1.205	-1.989 to -0.421	0.003
sCD40L, ng/mL	3.177	0.276 to 6.078	0.032

95% CI - 95% confidence interval; HDL - high-density lipoprotein; sCD40L - soluble CD40 ligand.

nary artery calcium score [28]. Nevertheless, theses scores do not assign a specific weight for each diseased segment and therefore do not account for the perfused territories supplied by stenoses located in different segments [12, 27, 28]. We used the Gensini score, which simultaneously measures coronary artery disease burden and considers the myocardium at risk for each lesion/segment [22]. This may explain our positive findings in contrast to those of the aforementioned studies. In fact, of the studies focusing on stable coronary artery disease, one reported increased sCD40L levels in multivessel disease compared to less severe disease and a positive correlation between sCD40L and the Gensini score [29]. Additional data on the relationship between stable coronary artery disease severity and sCD40L levels are scarce. On the other hand, to the best of our knowledge, there are no published data on the association between angina severity, as a measure of the clinical severity of CCS, and sCD40L. Therefore, our results may add to the knowledge on how inflammation may indirectly be reflected in clinical status.

Third, we describe for the first time the variation in sCD40L levels according to coronary revascularization status. In addition to having a causal role in atherosclerosis development, sCD40L might itself be influenced by atherosclerosis and reflect coronary artery disease burden and ischemic stress [30]. Indeed, in our sample, among patients with CCS, those with previous CABG showed lower levels of sCD40L than patients without previous CABG. It is known that severe chronic coronary

Table 6. Characteristic of patients with chronic coronary syndrome with and without prior coronary artery bypass grafting

	No previous CABG (n=45)	Previous CABG (n=23)	*p*-value
Clinical data			
Age, years	64.9 (10.2)	64.7 (7.3)	0.645
Male, n (%)	41 (91.1)	20 (87.0)	0.681
Hypertension, n (%)	43 (95.6)	21 (91.3)	0.599
Dyslipidemia, n (%)	42 (93.3)	23 (100.0)	0.546
Diabetes mellitus, n (%)	20 (44.4)	9 (39.1)	0.675
Smoking history (current or past), n (%)	26 (57.8)	11 (47.8)	0.436
Left ventricular ejection fraction > 50%, n (%)	45 (100.0)	23 (100.0)	-
Antiplatelet agent, n (%)	43 (95.6)	23 (100.0)	0.546
Statin therapy, n (%)	40 (88.9)	21 (91.3)	1.000
Laboratory data			
Hemoglobin, g/dL	13.6 (1.5)	13.7 (1.8)	0.780
Leukocyte count, 10^9/L	8.0 (1.8)	6.7 (1.6)	0.003
Neutrophil count, 10^9/L	4.8 (1.4)	3.8 (1.7)	0.001
Lymphocyte count, 10^9/L	2.2 (0.9)	1.8 (0.7)	0.133
Neutrophil/lymphocyte ratio	2.4 (1.0)	2.3 (1.1)	0.457
Platelet count, 10^9/L	233.6 (49.3)	199.7 (47.9)	0.011
Fasting glycaemia, mg/dL	113.7 (50.1)	110.5 (58.1)	0.892
Percentage of glycosylated hemoglobin	6.6 (1.5)	6.1 (1.3)	0.171
Creatinine, mg/dL	1.1 (0.4)	1.0 (0.3)	0.367
Total cholesterol, mg/dL	170.9 (42.6)	157.9 (51.6)	0.274
LDL-cholesterol, mg/dL	104.4 (35.5)	94.1 (41.6)	0.291
HDL-cholesterol, mg/dL	37.6 (8.9)	37.6 (9.6)	0.859
Triglycerides, mg/dL	155.6 (57.9)	136.0 (52.3)	0.169
C-reactive protein, mg/L	3.6 (1.3)	3.8 (1.9)	0.611
sCD40L, ng/mL	6.8 (3.5)	4.3 (2.1)	0.001
Anatomical data			
Nr. of vessels with obstructive disease	3.0 (0.9)	2.8 (0.7)	0.438
Nr. of obstructive lesions	4.1 (1.2)	3.8 (1.6)	0.243
Gensini score	71.1 (36.2)	76.3 (53.5)	0.928

Data are expressed as mean (SD), except if otherwise specified. CABG - coronary artery bypass grafting; HDL - high-density lipoprotein; LDL - low-density lipoprotein; Nr. - number; sCD40L - soluble CD40 ligand.

Table 7. Severity of native coronary artery disease according to time elapsed from coronary artery bypass grafting

	CABG 12 to 36 months before (n=12)	CABG at least 36 months before (n=11)	*p*-value
Nr. of vessels with obstructive disease	2.8 (0.7)	2.8 (0.8)	0.961
Nr. of obstructive lesions	3.8 (1.5)	3.8 (1.7)	0.920
Gensini score	68.8 (49.1)	84.5 (59.2)	0.493

Data are expressed as mean (SD). CABG - coronary artery bypass grafting; Nr. - number.

ischemia promotes the release of reactive oxygen species, cytokines and other inflammatory markers and that revascularization may ameliorate inflammation and vascular function [31, 32]. It is possible that the reduction of ischemic stress after bypass grafting may have reduced inflammation without modifying coronary atherosclerosis burden, which translated into

Table 8. Independent predictors of obstructive coronary artery disease excluding patients with prior coronary artery bypass grafting

Predictors	β	95% CI	*p*-value
Hypertension	0.058	0.007 to 0.464	0.007
HDL-cholesterol, mg/dL	0.893	0.828 to 0.964	0.004
sCD40L, ng/mL	1.344	1.016 to 1.778	0.038

95% CI - 95% confidence interval; HDL - high-density lipoprotein; sCD40L - soluble CD40 ligand.

lower sCD40L release into peripheral blood [21]. The hypothesis that sCD40L is a surrogate of coronary artery disease burden and myocardial ischemic stress needs to be explored in future studies. The scarce data available on the long-term effects of CABG on inflammation in patients with CCS point to an attenuation of the acute-phase reaction, reflected by a reduction in C-reactive protein levels [33]. Specifically, for sCD40L, the levels were reported to rise immediately after CABG, probably due to endothelial disruption and inflammatory activation related to the surgery itself, followed by a decrease in the first month after surgery [34]. Nevertheless, no published long-term data are available.

There are strengths of this study that should be acknowledged. The prospective nature of the study, the use of coronary computed tomography angiography to exclude coronary artery atherosclerosis in controls and, importantly, the multivariate analysis adjusting for confounders contributed to higher accuracy in the identification of sCD40L as a predictor of obstructive coronary artery disease compared to previous studies. In addition, analyses on the clinical and anatomical severity of CCS, with adjustment for confounding variables, added consistency to the results. To the best of our knowledge, the association between the clinical severity of CCS and sCD40L levels has not yet been reported. Finally, we describe for the first time a reduced expression of sCD40L in patients with prior coronary revascularization among those with obstructive coronary artery disease.

Our study has some limitations. First, this is a single-center study and additional studies from multiple centers are warranted. Second, we did not demonstrate a causal relationship between sCD40L and atherogenesis, which has already been studied [6, 35]. Instead, we observed that sCD40L probably mediates the interaction between inflammation and atherosclerotic disease expression, and may also be a surrogate marker. The consistency of our results, considering the multiple results pointing towards the same direction, after adjustment for confounders, reinforces the role of sCD40L as a mediator in this process.

In conclusion, the sCD40L levels were increased in patients with CCS, and sCD40L expression was associated with the clinical and anatomical severity of CCS, while coronary artery revascularization was associated with the attenuation of sCD40L dysregulation. Therefore, sCD40L may participate in the association between inflammation and atherosclerosis development, progression and clinical expression. The results of this study add to knowledge on the pathophysiology of coronary atherosclerosis. In addition, our findings suggest that sCD40L may be a promising complementary noninvasive tool for refining the stratification of coronary atherosclerosis burden and severity, which could contribute to the tailoring of the primary prevention strategies. These fields deserve further research.

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Disclosure of conflict of interest

None.

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