

## Soluble CD40L and Cardiovascular Risk in Asymptomatic Low-Grade Carotid Stenosis

Salvatore Novo, MD; Stefania Basili, MD; Rosalba Tantillo, MD; Angela Falco, MD; Valentina Davì, MD; Giuseppina Novo, MD; Egle Corrado, MD; Giovanni Davì, MD

**Background and Purpose**—We investigated whether soluble CD40L (sCD40L) may predict the risk of cardiovascular (CV) events in patients with asymptomatic carotid plaques.

**Methods**—Forty-two patients with asymptomatic low-grade carotid stenosis (ALCS) and 21 controls without any carotid stenosis were enrolled. All subjects had at least a major cardiovascular risk factor (CRF). Plasma levels of C-reactive protein (CRP), IL-6, and sCD40L were measured. Subjects were reviewed every 12 months (median follow-up, 8 years).

**Results**—ALCS patients had higher ( $P<0.0001$ ) CRP, IL-6, and sCD40L than controls. Fourteen patients experienced a CV event. Cox regression analysis showed that only high sCD40L levels ( $P=0.003$ ) independently predicted cardiovascular risk.

**Conclusions**—High levels of sCD40L may predict the risk of CV events in ALCS. (*Stroke*. 2005;36:673-675.)

**Key Words:** atherosclerosis ■ carotid stenosis ■ inflammation ■ risk factors

Carotid artery intima media thickness is associated with cardiovascular diseases (CVD).<sup>1</sup> Studies support the role of CD40–CD40 ligand interactions in atherothrombosis.<sup>2</sup> Patients with acute cerebral ischemia show upregulation of CD40 system.<sup>3</sup>

Cytokines and cytokine-inducible inflammatory molecules are regarded as predictive markers of cardiovascular events. Enhanced levels of C-reactive protein (CRP), IL-6, and soluble CD40 ligand (sCD40L) are associated with high risk of CVD.<sup>4,5</sup> We investigated whether these measurements may predict cardiovascular events in asymptomatic carotid plaques patients, beyond the current “standard” risk factors (CRF).

### Subjects and Methods

Subjects without previous CVD were examined for carotid atherosclerotic involvement because of presence of at least one CRF. Intima media thickness  $>1.5$  mm, acute and chronic inflammatory diseases, treatment with hormonal replacement therapy, steroids, aspirin, nonsteroidal anti-inflammatory drugs, or antioxidant supplements represented exclusion criteria.

Forty-two patients with asymptomatic low-grade carotid stenosis (ALCS) and 21 subjects without any carotid stenosis were consecutively enrolled (Table 1). All gave written informed consent. The study was approved by local ethics committee.

Patients were reviewed every 12 months or when they experienced cardiovascular events (myocardial infarction, angina, stroke, transient ischemic attack, carotid endarterectomy, intermittent claudication, need for percutaneous revascularization procedures or coronary artery bypass graft) (median follow-up, 8 years).

Blood samples were anticoagulated in Na citrate 3.8% (1:9 v:v), centrifuged at 1500g for 10 minutes, and plasma was stored at  $-80^{\circ}\text{C}$  until analysis.

IL-6 (R&D Systems) and sCD40L (Bender Medsystems) were measured by enzyme-linked immunosorbent assay; CRP by highly sensitive immunoassay (Abbot). Intra-assay and interassay coefficients of variation were  $<9\%$ .

The carotid arteries were evaluated with high-resolution B-mode ultrasonography using a 7.5-MHz duplex-type probe (Toshiba). Percent stenosis was graded as low-grade lumen stenosis because of plaque  $>15\%$  but  $<50\%$  (intima media thickness  $>0.85$  and  $<1.5$  mm). If no lesion was detected, subjects were considered as normal.

### Statistical Analysis

Statistical analysis was performed by  $\chi^2$  statistics, Pearson correlation coefficient, and by  $t$  test for independent samples. When necessary, appropriate nonparametric tests were used (Spearman correlation coefficient and Mann–Whitney  $U$  test). A multiple linear regression analysis was performed to further quantify the relationship between sCD40L and the variables in study. The clinical relevance of parameters for the prediction of all events and separately of combined endpoints myocardial infarction and stroke (hard endpoints) were estimated by univariate (by Log Rank Test) and multivariate (by Cox proportional hazard model including variables that achieved statistical significance in the univariate analysis) analyses.

Data are presented as mean (SD) or median (range) (SPSS by SPSS Inc and EGRET by SERC).

### Results

Patients and controls displayed comparable body mass index and lipid levels. In contrast, the prevalence of hypertension

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From the Department of Clinical Medicine and Emerging Pathologies (S.N., R.T., V.D., G.N., E.C.), University of Palermo, Italy; the Department of Medical Therapy (S.B.), University of Rome “La Sapienza,” Italy; and the Center of Excellence on Aging (A.F., G.D.), “G. d’Annunzio” University Foundation, University of Chieti, Italy.

Correspondence to Dr Giovanni Davì, “G. d’Annunzio” University Foundation, University of Chieti School of Medicine, Via Colle dell’Ara, 66013 Chieti, Italy. E-mail gdavi@unich.it

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**TABLE 1. Subject Characteristics**

Variable	Patients, n=42	<i>P</i>	Controls, n=21
Gender, M/F	26/16	>0.05	13/8
Age,* y	64±10	>0.05	61±11
Smokers,† no. (%)	5 (12)	>0.05	5 (24)
Hypertension,† no. (%)	29 (69)	0.007	7 (33)
Diabetes,† no. (%)	12 (29)	0.005	0 (0)
Body mass index,* kg/m <sup>2</sup>	26.9±3.3	>0.05	27.8±3.2
Total cholesterol,* mmol/L (mg/dL)	5.9±1.2 (228±48)	>0.05	5.9±1.3 (230±50)
Triglycerides,* mmol/L (mg/dL)	1.7±0.6 (154±66)	>0.05	1.5±0.7 (136±67)
HDL cholesterol,* mmol/L (mg/dL)	1.2±0.4 (47±14)	>0.05	1.2±0.3 (46±13)
LDL cholesterol,* mmol/L (mg/dL)	3.9±1.1 (150±42)	>0.05	4.0±1.1 (156±42)
IL-6,‡ pg/mL	2.1 (0.5–6.1)	<0.0001	0.5 (0.5–2.0)
C-reactive protein,‡ mg/L	2.0 (0.1–5.2)	<0.0001	0.7 (0.1–2.8)
sCD40L,‡ ng/mL	6.2 (1.4–15.8)	<0.0001	1.4 (0.5–4.5)

\*Mean±SD, *t* test for independent samples.† $\chi^2$  test.‡Median (range), Mann-Whitney *U* test.

and diabetes was increased in ALCS (Table 1). ALCS had higher ( $P<0.0001$ ) CRP, IL-6, and sCD40L than controls (Table 1). This difference was still significant after patients' stratification according to CRF presence, mainly hypertension and diabetes.

Among ALCS, sCD40L directly correlated with CRP ( $R_s=0.59$ ;  $P<0.0001$ ) and IL-6 ( $R_s=0.63$ ;  $P<0.0001$ ). Multiple regression analysis indicated that IL-6 ( $\beta=0.41$ ,  $SE=0.16$ ;  $P<0.02$ ) and CRP ( $\beta=0.36$ ,  $SE=0.15$ ;  $P<0.03$ ) predicted sCD40L levels, independently of CRF.

Median follow-up was 3225 (37 to 3681) days. No patient was lost to follow-up. Fourteen (33%) patients experienced vascular events (4 nonfatal myocardial infarctions, 2 strokes, 4 transient ischemic attacks, 2 intermittent claudications, and 2 had need for percutaneous revascularization procedures). They had higher median values of sCD40L (9.1 versus 5.6 ng/mL;  $P<0.02$ ) than those who remained event-free.

Of the parameters listed in Table 2, only high sCD40L levels entered the Cox proportional hazard model ( $P=0.003$ ) (Figure). A separate analysis for hard endpoints ( $n=6$ ) showed that only sCD40L high values identified patients who experienced critical events (low values=0% versus high values=43%; log-rank test, 17.30;  $P<0.001$ ).

## Discussion

Prospective studies showed that CRP, IL-6, and CD40L predict the risk of CVD,<sup>4,5</sup> and plasma levels of both CRP and IL-6 are independently related to traditional cardiovascular risk factors in women.<sup>6</sup> Furthermore, sCD40L predicts patients with features of high-risk plaques<sup>7</sup> and an association between CRP and carotid atherosclerosis has been observed in women.<sup>8</sup>

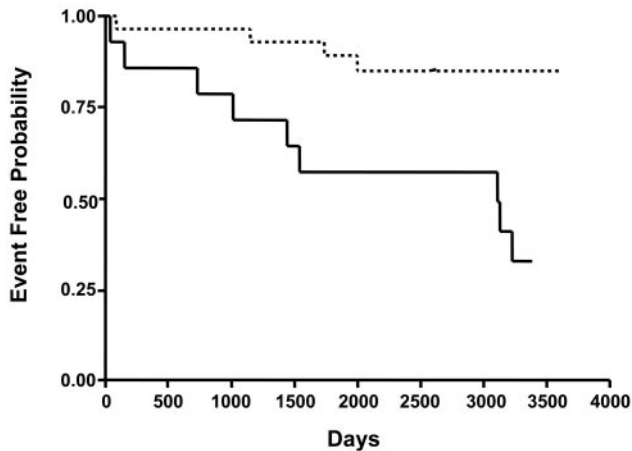
CD40L may play a pathogenetic role in atherosclerosis. CD40L is rapidly upregulated during platelet activation and triggers an inflammatory response in cells that constitutively express CD40, ie, endothelial cells and monocytes.<sup>9</sup> CD40 and CD40L are both overexpressed in human and experimental atherosclerotic lesions, particularly in advanced, rupture-prone plaques.<sup>10</sup> Accumulation of

T lymphocytes in the fibrous cap may induce macrophages to secrete metalloproteinases via CD40, inducing plaque instability.<sup>11</sup> Conversely, CD40L induces tissue factor expression,<sup>10</sup> accounting for thrombotic events within the plaque.

**TABLE 2. Univariate Analysis**

Variables	No. of Patients	% Endpoint	Log-Rank Test	<i>P</i>
Gender			1.827	>0.05
Females	16	19		
Males	26	42		
Smoking			0.329	>0.05
No	37	32		
Yes	5	40		
Hypertension			0.785	>0.05
No	13	23		
Yes	29	38		
Diabetes Mellitus			0.002	>0.05
No	30	33		
Yes	12	33		
Hypercholesterolemia			0.821	>0.05
No	19	26		
Yes	23	39		
CRP			0.192	>0.05
Low	33	36		
High	9	22		
IL-6			0.882	>0.02
Low	21	24		
High	21	43		
sCD40L			11.30	<0.001
Low	28	18		
High	14	64		

Low indicates below and High indicates above median of 42 ALCS patients.



Kaplan–Meier curves for ALSC patients grouped by their levels of sCD40L (Cox proportional hazard model, hazard ratio: 5.96;  $P=0.003$ ). Lines represent subjects with sCD40L above median (solid line,  $n=14$ ) or below median (broken line,  $n=28$ ) of patients.

We found increased circulating IL-6, CD40L, and CRP in ALSC and a strong association among these parameters. In the multivariate analysis, only sCD40L above median predict the risk of cardiovascular events, even after adjustment for IL-6, CRP, and CRF. This is consistent with the finding that high CD40L indicates increased risk of coronary events in unstable angina.<sup>12</sup>

Our data confirm that CD40–CD40L interactions represent a main molecular mechanism linking inflammation and thrombosis. Inhibition of this system may represent a potential therapeutic target capable of inducing a more stable carotid plaque phenotype.

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