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The relationship between soluble CD40 ligand levels and Framingham coronary heart disease risk score in healthy volunteers

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Abstract

Elevated soluble CD40 ligand (sCD40L) levels are associated with an increased risk of cardiovascular events in patients with acute coronary syndromes and in middle-aged healthy women. However, the relationship between sCD40L and global risk assessment remains unclear. The present study was designed to examine the relationship between sCD40L and Framingham Coronary Heart Disease Risk Scores (FCRS) in healthy middle-aged men. The study population consisted of 400 active and retired male firefighters, with no previous history of cardiovascular disease, as part of the *Firefighters and Their Endothelium* (FATE) study. FCRS correlated poorly with sCD40L levels ($p = 0.14$). Soluble CD40L concentrations correlated only with total ($r = 0.105$; $p = 0.035$) and LDL cholesterol ($r = 0.104$; $p = 0.039$), and CRP levels ($r = 0.11$; $p = 0.03$). Compared with participants with sCD40L levels <4.36 ng/mL (75th percentile), participants with sCD40L levels >4.36 ng/mL had higher total ($p = 0.016$) and LDL cholesterol ($p = 0.018$), CRP levels ($p = 0.034$) and FCRS ($p = 0.012$). Multivariate analysis revealed that CRP level was the only parameter that independently correlated with the sCD40L levels ($p = 0.032$). This is the first study to evaluate the relationship between sCD40L levels and Framingham global risk assessment in a large cohort of otherwise healthy individuals. We demonstrate that sCD40L levels poorly correlate with both the individual components and the calculated FCRS. Long-term follow-up of the FATE study will shed light on whether the predictive value of sCD40L is independent of Framingham based global risk assessment.

Keywords: Atherosclerosis; C-reactive protein; Inflammation; Risk factors; Soluble CD40 ligand

1. Introduction

Increasingly, it has been realized that vascular inflammation plays a key role in atherosclerotic lesion formation, progression and eventual rupture [1]. Accordingly, markers of inflammation and endothelial activation have become useful by providing additional information about cardiovascular risk and prognosis, as well as providing new targets for treatment [2,3].

The interaction of CD40 receptor (CD40) and its counterpart CD40 ligand (CD40L) was originally identified to be a critical system in cellular immunity and inflammation [4]. Increasing evidence suggests that the CD40/CD40L system plays an important part in cardiovascular disease progression and atherosclerotic plaque destabilization [4,5]. CD40L exists in a soluble form (sCD40L), which is shed from a variety

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of activated cells and is biologically active. Elevated sCD40L is associated with an increased risk of cardiovascular events in patients with acute coronary syndromes [6,7]. Furthermore, apparently healthy women with increased plasma levels of soluble CD40L have also been shown to be at increased risk of cardiovascular events [8].

Traditionally, the Framingham Coronary Heart Disease Risk Score (FCRS) is used for clinicians to estimate 10-year risk for cardiovascular disease in middle-aged individuals [9]. Despite the value of FCRS as a measure of global risk, it has become increasingly clear that this measure of risk assessment may not capture a large number of patients of risk of atherosclerotic events [10]. Indeed, recent studies demonstrate that the powerful predictive value of C-reactive protein (CRP) may be over and above FCRS assessment [11].

There are very few data available directly comparing sCD40L levels with calculated FCRS in healthy individuals [8,12]. Although sCD40L levels stratify risk of future cardiovascular events, the extent to which sCD40L level reflects any individual component of the FCRS is unclear. Such an evaluation is essential to appreciate the potential value of evaluating sCD40L as an adjunctive biomarker of cardiovascular disease.

2. Methods

The study was performed by analyzing a subgroup in the *Firefighters and Their Endothelium* (FATE) study, an ongoing prospective, longitudinal study of 1585 middle-aged active and retired firefighters across Canada designed to assess the value of brachial artery flow-mediated vasodilation as a predictor of cardiovascular events. The details of objectives and design have been described elsewhere [13]. Participants were not eligible if they had documented coronary artery disease, cerebral vascular disease or peripheral vascular disease. From the FATE population, we randomly selected 400 participants at different quartiles of FCRS (Table 1) to evaluate the relationship between sCD40L levels and components of the FCRS score. Within each of the quartiles of risk, patient identification numbers were selected by random number generation obtaining a sample of 400 spread over the range of FCRS. The selections were made independently of knowledge of any other factors other than the FCRS. FCRS were calculated as previously suggested by Grundy et al. [14].

2.1. Biochemical analysis

Fasting blood samples of plasma were obtained at baseline and stored in −80 °C until analyzed. Soluble CD40 ligand concentrations were measured by ELISA (Alexis Biochemicals). Analysis was performed in a blinded fashion. Intra-assay coefficient of variation among the triplicates for all samples was 4%. High sensitive-CRP concentrations were measured by a particle-enhanced immunoturbidimetric method with the use of a Hitachi 912 analyzer (Roche Diagnostics) and reagents of Tina-quant C-reactive protein [latex] ultra sensitive assay (Roche Diagnostics). This measurement was standardized against International Federation of Clinical Chemistry Certified Reference Material Standard (IFCC CRM 470). The lower detection limit reported for the assay was 0.21 mg/L and the coefficient of variation at 0.21 mg/L was an acceptable 7.2%. Lipid levels were measured in a laboratory that participates in the Centers for Disease Control standardization.

2.2. Statistical analysis

The data are expressed as the mean value ± S.D. Multivariate analysis was performed with stepwise linear regression model with controlling for potential confounders and known cardiovascular risk factors. All analyses were performed with SAS software Version 8. Statistical significance was defined as a two-sided p-value <0.05.

3. Results

The study population consisted of 400 middle-aged healthy male volunteers, with no previous history of cardiovascular disease. The average concentration of sCD40L in this population was 3.43 ± 1.65 ng/mL (median 3.32 ng/mL).

Table 1
Distribution of cardiovascular risk factors, C-reactive protein and sCD40L concentrations according to 10-year risk for coronary heart disease

Variables	10-year risk for coronary heart disease				$p$for trend
	<3% (n = 95)	4–5% (n = 76)	6–9% (n = 101)	>10% (n = 119)	
Age (year)	38 (37–40)	45 (44–46)	50 (49–51)	57 (56–59)	<0.001
Systolic blood pressure (mmHg)	119 (117–121)	122 (119–125)	128 (125–131)	141 (138–144)	<0.001
Diastolic blood pressure (mmHg)	78 (77–80)	81 (78–83)	81 (80–83)	85 (83–87)	<0.001
Total cholesterol (mmol/L)	4.7 (4.5–4.9)	5.1 (4.9–5.2)	5.5 (5.3–5.7)	5.7 (5.5–5.8)	<0.001
HDL cholesterol (mmol/L)	1.4 (1.3–1.4)	1.2 (1.2–1.3)	1.2 (1.1–1.2)	1.2 (1.1–1.2)	<0.001
LDL cholesterol (mmol/L)	2.8 (2.7–3.0)	3.2 (3.0–3.3)	3.5 (3.3-3.6)	3.6 (3.5–3.7)	<0.001
Triglyceride (mmol/L)	1.1 (1.0–1.2)	1.4 (1.2–1.5)	1.9 (1.7–2.1)	1.9 (1.7–2.0)	<0.001
Fasting glucose (mmol/L)	5.1 (4.9–5.3)	5.2 (5.1–5.3)	5.3 (5.2–5.3)	5.5 (5.4–5.7)	<0.001
Smoke (%)	1.0	6.6	16.5	19.2	<0.001
C-reactive protein (mg/dL)	1.7 (1.0–2.4)	1.5 (0.9–2.0)	1.9 (1.4–2.4)	2.8 (2.0–3.5)	0.027
sCD40L (ng/mL)	3.26 (2.98–3.55)	3.28 (2.92–3.65)	3.52 (3.15–3.88)	3.61 (3.30–3.92)	0.14

Data are shown as mean (95% CI); sCD40L, soluble CD40 ligand.