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# Transcoronary Concentration Gradient of sCD40L and hsCRP in Patients with Coronary Heart Disease

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## Summary

*Background:* Recent studies indicated that local inflammation played a pivotal role in the pathogenesis of coronary heart disease. Soluble CD40 ligand (sCD40L) and hsC- reactive protein (hsCRP) are important inflammatory mediators. However, whether they can reflect local coronary inflammation is unclear.

*Hypothesis:* We hypothesized that transcoronary concentration gradient of sCD40L could reflect local inflammation in patients with coronary heart disease (CHD) more reliably.

*Methods:* Forty subjects were divided into unstable angina pectoris (UAP) group ($n = 20$), stable angina pectoris (SAP) group ($n = 10$), and controls ($n = 10$). Blood samples were collected from the coronary sinus (CS), aortic root (AO), and femoral vein (FV). The coronary circulation was expressed as CS-AO difference, while system circulation was expressed as FV-AO difference. sCD40L and hs-CRP were measured.

*Results:* Complex lesions were more frequent in the UAP group than in the SAP group (85% vs. 40%, $p<0.05$). CS-AO differences of sCD40L were much greater in the UAP group than in the SAP or control groups, and were greatly higher than FV-AO difference in UAP group ($465.49 \pm 247.85$ pg/mL vs. $-14.94 \pm 83.41$ pg/mL; $465.49 \pm 247.85$ pg/mL vs. $-7.66 \pm 78.54$ pg/mL; $465.49 \pm 247.85$ pg/mL vs. $-7.99 \pm 141.34$ pg/mL, all $p<0.001$). CS-AO differences of sCD40L were higher in patients with complex lesions than with smooth lesions ($657.86 \pm 384.76$ pg/mL vs. $317.62 \pm 409.98$ pg/mL, $p<0.01$). There were no significant differences of CS-AO in hs-CRP among the three groups.

*Conclusions:* In patients with CHD, the transcoronary concentration gradient of sCD40L is more sensitive than hsCRP, and sCD40L possibly a better marker of local inflammtion and plaque instability.

**Key words:** soluble CD40 ligand, HsC-reactive protein, coronary disease, inflammation

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## Introduction

It has become evident that inflammation plays an important role in all stages of the atherosclerotic disease process, from lesion initiation to progression and, ultimately, to plaque rupture. Soluble CD40 ligand (sCD40L) and hsC-reactive protein (hsCRP) that appear to be linked to inflammation and atherogenesis have been identified. Hs-CRP was found to be associated with an increased risk of cardiovascular events in the asymptomatic population and in patients with unstable angina pectoris (UAP) and stable angina pectoris (SAP).[1,2]

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CD40L is a trimeric, transmembrane protein of the tumor necrosis factor family and together with its receptor CD40 is an important contributor to the inflammatory processes leading to atherosclerosis, plaque destabilization, and thrombosis.[3,4] Studies showed that sCD40L was increased in unstable angina,[5] hypercholesterolemia[6] and was an independent prognostic marker for cardiovascular diseases among healthy individuals.[7]

Since the pathophysiological changes in patients with coronary artery disease mainly occur in the coronary circulation, the peripheral vein blood cannot fully reflect these changes. Thus, measuring concentrations of inflammatory markers in peripheral vessels and single blood samples may not fully reflect low-grade inflammatory activity in atherosclerotic coronary arteries.

We hypothesized that transcoronary concentration of sCD40L and hsCRP levels may identify patients with coronary heart disease (CHD) more effectively. Therefore we investigated the changes of transcoronary concentration gradient of sCD40L, hsCRP in patients with CHD and compared with normal subjects in order to find a better biomarker reflecting local coronary inflammation.

## Methods

### Patients

From December 2002 to June 2004, forty subjects from the Department of Cardiology, Qilu Hospital, Shandong University were enrolled in the study. According to the clinical diagnosis and coronary angiography, subjects were divided into UAP, SAP, and control groups. The UAP group consisted of 20 patients who had anginal episodes at rest or angina during a mild degree of effort within 48 hours of the study without a significant increase in CK-MB levels. The SAP group consisted of 10 patients with typical effort angina or positive treadmill exercise testing but no episodes of angina at rest.[8] The control group consisted of 10 patients with chest pain syndrome ($n = 8$) or paroxysmal supraventricular tachycardia under frequency ablation ($n = 2$). None of them had a visible coronary artery stenosis. Exclusion criteria were as follows, body temperature $>38.0\,^{\circ}$C, severe heart failure (killip class 3 or 4 and NYHA class III or IV), inflammatory diseases (e.g., infections, autoimmune diseases), malignancies, impaired liver function, renal failure, and recent major surgery. Informed consent was obtained from all subjects based on a protocol approved by the Ethics Committee of QiLu Hospital, Shandong University.

### Coronary Angiography

Coronary angiography was performed in all subjects. Significant coronary artery stenosis was defined as at least 75% reduction in the internal diameter of the right, left anterior descending, or left circumflex coronary arteries and their branches, or $\geqslant$50% reduction in the internal diameter of the left main trunk.[9] Coronary arteries were considered angiographically normal if they had no appreciable stenosis. The morphologic appearance of each lesion, classified as complex or smooth, was independently assessed by 2 investigators at separate sittings using a previously described method.[9,10]

Complex stenoses were defined by the presence of one or more of the following criteria: (i) irregular or scalloped borders, (ii) abrupt lesion edges perpendicular to or overhanging the vessel wall, (iii) ulceration, or (iii) the presence of a filling defect consistent with thrombus. The presence of haziness of the lumen border was not sufficient by itself to define complexity. Stenoses without these features were categorized as smooth lesions.[11]

### Biochemical Analysis

Before the injection of a contrast medium, blood samples from the coronary sinus (CS), aortic root (AO), and femoral vein (FV) were drawn at the emergency department in patients with UAP, SAP and controls. The plasma was stored at $-80^{\circ}$C until assayed. Plasma level of sCD40L was determined by ELISA kit (R&D Systems) according to the manufacturer's instructions. Hs-CRP was measured at 550 nm by a particle enhanced immunoturbidimetric assay (Orine Diagnostica) on a Hitachi 7170A analyzer.

### Statistical Analysis

Statistical evaluation was performed with SPSS10.0 software. Numerical data were expressed as mean $\pm$ S.D, categorical variables as number (%). Continuous variables were compared by means of one-way analysis of variance with Scheffe posteriori comparisons and student's $t$ test as appropriate. Chi square test was used for categorical variables. $p<0.05$ was considered statistically significant.

## RESULTS

### Patient Characteristics

The three groups were well matched in terms of age and gender. Compared with controls, hypertension and diabetes were more prevalent in UAP and SAP groups, and low density lipoprotein-cholesterol (LDL-C) was higher in UAP and SAP groups, with no difference between UAP and SAP groups (Table 1).

### Angiographic Data

Coronary angiograms showed that UAP group had a significantly higher prevalence of complex lesions (85%) than the SAP group (40%) ($p<0.05$) (Figure1).

TABLE 1 Clinical characteristics in three groups of subjects

| | Controls ($n = 10$) | SAP group ($n = 10$) | UAP group ($n = 20$) |
|---|---|---|---|
| Age (y) | 51.37 ± 7.82 | 58.84 ± 8.24 | 62.28 ± 6.23 |
| Men n [%] | 5 (50) | 6 (60) | 12 (60) |
| Hypertension n [%] | 0 | 8 (80)* | 16 (60)* |
| Diabetes n [%] | 0 | 6 (60)* | 11 (55)* |
| Smoking n [%] | 4 (40) | 4 (40) | 7 (35) |
| TC (mmol/L) | 4.75 ± 0.81 | 5.35 ± 0.92 | 5.52 ± 0.29 |
| TG (mmol/L) | 1.71 ± 0.92 | 1.65 ± 0.63 | 1.94 ± 0.75 |
| LDL-C (mmol/L) | 2.62 ± 0.57 | 3.55 ± 0.49* | 3.79 ± 0.94* |
| HDL-C (mmol/L) | 1.24 ± 0.38 | 1.09 ± 0.35 | 0.91 ± 0.20 |
| GLu (mmol/L) | 5.26 ± 0.75 | 5.78 ± 0.83 | 5.99 ± 0.88 |
| WBC ($10^9$/L) | 6.46 ± 1.25 | 6.25 ± 1.41 | 7.22 ± 1.79 |
| N (%) | 58.74 ± 11.27 | 60.58 ± 17.42 | 70.25 ± 11.42 |
| Medication (%) | | | |
| Aspirin | 0 | 6 (60) | 14 (70) |
| Statin therapy | 0 | 2 (20) | 6 (30) |
| β-Blockers | 0 | 9 (90) | 15 (75) |
| ACE inhibitors | 0 | 8 (80) | 12 (60) |
| No. of diseased vessels (%) | | | |
| 1 | 0 (0) | 2 (20) | 4 (20) |
| 2 | 0 (0) | 4 (40) | 7 (35) |
| 3 | 0 (0) | 4 (40) | 9 (45) |

Abbreviations: TC = total cholesterol, TG = triglyceride, HDL-C = high density lipoprotein, WBC = white blood count, N (%) = neutrophil percentage, N = neutrophil count.
* $p < 0.05$ compared with controls.

**Comparison of Plasma sCD40L and hsCRP**

As shown in Table 2, sCD40L was significantly higher in SAP and UAP groups compared with controls, with a significant difference between UAP and SAP group. In the UAP group, sCD40L was higher in CS than in FV and AO. Hs-CRP was significantly higher in UAP group compared with controls and SAP group. No significant change of hsCRP was found between controls and the SAP group.

Investigation of sCD40L and hs-CRP in different coronary lesions revealed sCD40L was higher in patients with complex lesions compared with patients with smooth lesions. There was no significant difference of hs-CRP between patients with smooth lesions and complex lesions (Table 3).

**Changes of sCD40L and hsCRP between coronary circulation and systemic circulation**

In the UAP group, CS-AO difference in sCD40L was significantly higher than FV-AO difference. There were no differences in sCD40L between coronary circulation

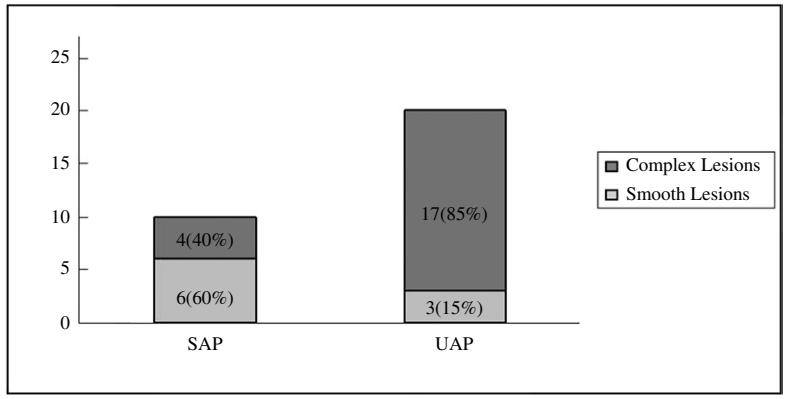


FIG. 1 Comparison of the angiographic morphologies of the culprit lesions between the SAP and UAP groups. 85% patients of UAP group demonstrated complex lesions compared with only 40% patients of SAP group ($p < 0.05$).

TABLE 2 Comparison of sCD40L and hs-CRP in controls and CHD groups

| | Controls ($n = 10$) | SAP group ($n = 10$) | UAP group $n = 20$ |
|---|---|---|---|
| sCD40 (pg/mL) | | | |
| FV | $2200.65 \pm 567.86$ | $3108.56 \pm 642.89^{**}$ | $5516.99 \pm 731.49^{***}\Delta\Delta$ |
| AO | $2209.32 \pm 578.58$ | $3124.69 \pm 610.07^{*}$ | $5524.98 \pm 718.85^{***}\Delta\Delta$ |
| CS | $2206.57 \pm 591.47$ | $3111.17 \pm 599.13^{**}$ | $5990.46 \pm 704.54^{***}\Delta\Delta\dagger$ |
| CS-AO | $-7.66 \pm 78.54$ | $-14.94 \pm 83.41$ | $465.49 \pm 247.85^{***}\Delta\Delta\S$ |
| FV-AO | $-8.44 \pm 69.45$ | $-17.22 \pm 97.64$ | $-7.99 \pm 141.34$ |
| hsCRP (pg/mL) | | | |
| FV | $2.28 \pm 0.82$ | $2.96 \pm 1.04$ | $5.63 \pm 1.41^{**}\Delta$ |
| AO | $2.42 \pm 0.86$ | $2.84 \pm 1.02$ | $5.72 \pm 1.47^{**}\Delta$ |
| CS | $2.40 \pm 0.91$ | $2.93 \pm 1.16$ | $5.69 \pm 1.31^{**}\Delta$ |
| CS-AO | $-0.03 \pm 1.39$ | $0.10 \pm 2.55$ | $-0.04 \pm 0.88$ |
| FV-AO | $-0.12 \pm 1.68$ | $0.12 \pm 2.37$ | $-0.08 \pm 0.46$ |

$^{*}$ $p < 0.05$,
$^{**}$ $p < 0.01$,
$^{***}$ $p < 0.001$ compared with controls; $\Delta$ $p < 0.05$, $\Delta\Delta$ $p < 0.001$ compared with SAP group; $\dagger$ $p < 0.05$ compared with FV and AO in UAP group; § $p < 0.001$ compared with FV-AO in UAP group.

and system circulation in the SAP group and controls. Hs-CRP levels showed no significant differences between coronary circulation and system circulation among three groups (Table 2, Figure 2).

Level of sCD40L in coronary circulation was significantly higher than in system circulation in the complex lesions group. However, hs-CRP showed no difference in lesions between coronary circulation and system circulation (Table 3, Figure 3).

TABLE 3 Comparison of sCD40L and hs-CRP in different coronary lesions

| | Smooth lesions ($n = 9$) | Complex lesions ($n = 21$) |
|---|---|---|
| sCD40 (pg/mL) | | |
| FV | $3361.33 \pm 1165.87$ | $5165.40 \pm 1171.11^{**}$ |
| AO | $3436.21 \pm 1141.59$ | $5120.02 \pm 1137.37^{***}$ |
| CS | $3753.89 \pm 1373.48$ | $5777.89 \pm 1072.62^{**}$ |
| CS-AO | $317.62 \pm 409.98$ | $657.86 \pm 384.76^{*}$ |
| FV-AO | $-5.17 \pm 102.74$ | $-13.59 \pm 138.03$ |
| hsCRP (pg/mL) | | |
| FV | $4.02 \pm 2.13$ | $5.05 \pm 1.61$ |
| AO | $3.95 \pm 2.17$ | $5.11 \pm 1.72$ |
| CS | $4.07 \pm 2.18$ | $5.07 \pm 1.60$ |
| CS-AO | $0.45 \pm 2.41$ | $-0.18 \pm 1.10$ |
| FV-AO | $0.17 \pm 2.04$ | $-0.93 \pm 1.03$ |

$^{*}$ $p < 0.01$,
$^{**}$ $p < 0.01$ compared with smooth lesion group;
$^{***}$ $p < 0.001$ compared with smooth lesion group.

## Discussion

The present study demonstrated that sCD40L, not hsCRP, exited transcoronary concentration gradient in the UAP group and in patient with complex lesions.Our study extended the previous observations that sCD40L possibly become a more sensitive marker of local inflammation and plaque instability than hsCRP.

It has become increasingly recognized that atherosclerosis is a chronic inflammatory process, characterized by the accumulation of lipid, inflammatory cells and necrotic material within the arterial wall. Local inflammation plays an important role in the pathogenesis of atherosclerosis, from lesion initiation to progression and, ultimately, to plaque rupture. As a result, it is of great interest to identify a sensitive and reliable biomarker reflecting local coronary inflammation.

High levels of sCD40L had been shown to be associated with cardiovascular events[12,13] and identified patients with acute coronary syndromes (ACS) at heightened risk of death and recurrent myocardial infarction (MI).[14] Our data were consistent with previous studies[5,15] that sCD40L was significantly higher in patients with UAP, and levels of sCD40L were higher in coronary circulation than in system circulation. But the most valuable findings of this study were that sCD40L was higher in patients with complex lesions than with smooth lesions, suggesting sCD40L might correlate with plaque instability.

Most studies have focused on the changes of systemic inflammatory factors levels in CHD. However, elevations of these markers can be nonspecific and do not reflect focal inflammatory activity in the coronary arteries. Thus, measuring concentrations of inflammatory markers in peripheral vessels and single blood samples may not fully reflect low-grade inflammatory activity in atherosclerotic coronary arteries. In order to explore the changes of inflammation factor in transcoronary circulation we used CS-AO difference to represent coronary

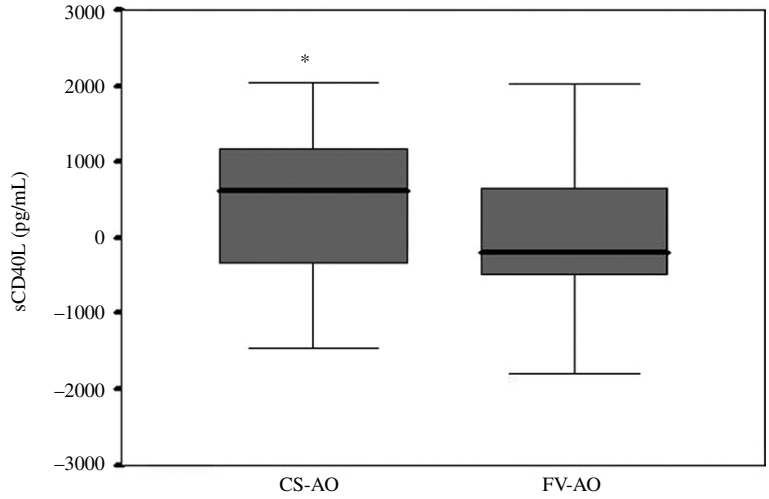


FIG. 2 Comparison of sCD40L in coronary circulation and system circulation in UAP group. $^{*}p < 0.001$ compared with FV-AO.

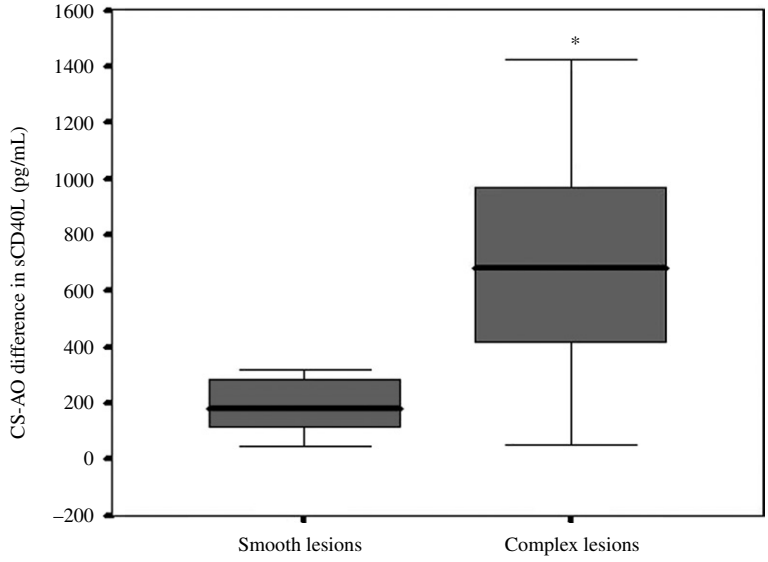


FIG. 3 Comparison of sCD40L in coronary circulation in different coronary lesions. $^{*}p < 0.05$ compared with smooth lesions group.

circulation concentration gradient and FV-AO difference for systemic circulation concentration gradient.

Complex lesions are frequent in patients with unstable angina and probably represent ruptured atherosclerotic plaques or partially occlusive thrombi, or both.[11] In the present investigation, the presence of CS-AO difference in sCD40L was significantly higher than FV-AO difference in UAP group. Furthermore, CS-AO difference in sCD40L was also higher in patients with complex lesions. A recent review demonstrated that platelets are the primary source of circulating sCD40L.[16] In patients with UAP, inflammatory damage to plaque may result in platelet adhesion and aggregation and also T cell activation, which are major contributors to comparable acute increase in sCD40L levels. It suggests that sCD40L may reflect local inflammation in atherosclerotic coronary arteries and may be involved in the vulnerability of plaques. The exact mechanism is unclear, but it might be explained by the severity of local inflammation. Further investigation is needed to clarify the precise mechanisms of sCD40L in the pathogenesis of complex lesions.

C-reactive protein is an acute-phase reactant that is produced in response to inflammatory stimuli. It has been shown to be a reliable measure of underlying systemic inflammation and a predictor of future cardiovascular events.[2] Translesional CRP gradient may be the powerful indicator of a vulnerable plaque.[17] However, the present study found that there was no difference of hs-CRP in coronary circulation compared to system circulation, and no significant difference in different lesion groups.

This indicated hs-CRP might not fully reflect low-grade inflammatory activity in atherosclerotic coronary arteries. The apparent inconsistency in our results may be the result of the different sites of sample collection and patients included.

In conclusion, transcoronary of sCD40L level collected in CHD patients seemed to be more sensitive as a tool for specific marker of local inflammation and plaque instability. Measuring sCD40L might provide more information in diagnosing and managing CHD patients.

The limitation of this study was the relatively small number of patients enrolled and troponin was not measured as a diagnostic marker. Further studies are needed to illustrate the clinical use of transcoronary of sCD40L independently or in combination with other markers in the prediction of cardiovascular events.

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