Serial Changes in Circulating Concentrations of Soluble CD40 Ligand and C-Reactive Protein in Patients With Unstable Angina Undergoing Coronary Stenting

Role of Inflammatory Mediators in Predicting Late Restenosis

Hon-Kan Yip, MD; Chiung-Jen Wu, MD; Cheng-Hsu Yang, MD; Hsueh-Wen Chang, PhD*; Chih-Yuan Fang, MD; Wei-Chin Hung, MD; Chi-Ling Hang, MD

Background This study tested the hypothesis that serum concentrations of high-sensitivity C-reactive protein (hs-CRP) and soluble CD40 ligand (sCD40L) significantly reflect serial changes in patients with unstable angina, and thus the serum concentrations of these inflammatory biomarkers may be good candidates for predicting late restenosis after coronary stenting.

Methods and Results The circulating concentrations of sCD40L and hs-CRP were prospectively measured (both pre-procedure, and on days 21, 90, and 180 after the procedure) in 77 consecutive patients with unstable angina undergoing coronary stenting. These inflammatory mediators were also evaluated in 30 healthy volunteers. The serum concentrations of sCD40L and hs-CRP were significantly higher pre-procedure in study patients than in normal control subjects (all p values <0.0001). These inflammatory markers then declined to a substantially lower concentration by day 21 (all p values <0.05). Circulating concentrations of hs-CRP in each patient then differed little from each other afterwards. However, the sCD40L concentration was once again raised significantly on days 90 and 180 as compared to day 21 (both p values <0.05). This study found no significant link between raised circulating concentrations of sCD40L and hs-CRP and late restenosis.

Conclusions Circulating concentrations of sCD40L and hs-CRP were significantly increased in unstable angina patients pre-procedure and declined substantially thereafter. However, the circulating concentrations of these 2 inflammatory mediators were not useful in predicting late restenosis following coronary stenting.

Key Words: Coronary stenting; Inflammatory mediators; Restenosis; Unstable angina
Methods

Patient Population and Inclusion Criteria
The present study enrolled consecutive patients admitted to hospital for unstable angina between March 2003 and January 2004. For the purposes of the present study, the serum concentrations of sCD40L and high-sensitivity (hs) CRP of all patients who underwent coronary stenting (all with bare metal stents) were prospectively measured. Venous blood samples were drawn before coronary angiographic study in the cardiac catheterization room (acute phase), and then on days 21 (convalescent phase), 90 (hyperplasia phase), and 180 (chronic phase) after the procedure. To circumvent other potential influences on the serum concentrations of sCD40L and hs-CRP, we excluded patients with a history of recent surgery or trauma during the preceding 2 months, those who had renal insufficiency (creatinine >1.5 mg/dl), malignancy or liver cirrhosis, febrile disorders, acute or chronic inflammatory disease on study enrolment, >1.5 mg/dl), malignancy or liver cirrhosis, febrile disorders, acute or chronic inflammatory disease on study enrolment, acute myocardial infarction (AMI) onset of <3 months, or had had a procedure-related myocardial infarction (MI). Over a 10-month period, 80 consecutive patients with unstable angina were recruited and underwent stenting on a planned basis. Restenosis was defined as the presence of recurrent ischemia, new onset of AMI, repeated target vessel revascularization, or 30-day death. Multi-vessel disease was defined by stenoses of >50% in ≥2 major epicardial coronary arteries. Restenosis was defined as the presence of ≥50% diameter stenosis in the dilated segment. Unstable angina was defined by using Braunwald's classification.19

In our laboratory, the mean intra-assay coefficients of variance were 3.21 and 2.96%, respectively. The concentration of sCD40L in plasma was determined in duplicate by using a standard enzyme-linked immunosorbent assay and a commercial kit (R and D Systems; Minneapolis, MN, USA) according to the description of the manufacturer. Intra-individual variability of sCD40L concentrations was assessed in the study patients, risk-control subjects, and healthy subjects. In our laboratory, the mean intra-assay coefficient of variance was <7%.

Angiographic Analysis, Definitions and Data Collection
Quantitative angiographic analysis of the percentage of minimal lumen diameter (MLD) stenosis, lesion length and reference lumen diameter (RLD) was performed by using a digital edge-detection algorithm (DUQUE System)18 and choosing end-diastolic frames demonstrating the stenosis in its most severe and non-foreshortened projection. With the contrast-filled guiding catheter used as the calibration standard, the reference and MLDs were calculated before and after stenting.

A combined 30-day major cardiac event was defined as recurrent ischemia, new onset of AMI, repeated target vessel revascularization, or 30-day death. Multi-vessel disease was defined by stenoses of >50% in ≥2 major epicardial coronary arteries. Restenosis was defined as the presence of ≥50% diameter stenosis in the dilated segment. Unstable angina was defined by using Braunwald’s classification19.

Detailed in-hospital and outpatient department data were obtained, including age, sex, coronary risk factors, medical history, characteristics of chest pain, electrocardiographic findings, creatinine concentration, white blood cell count, cardiac isoenzymes and troponin concentration before and after cardiac catheterization, angiographic findings, and number of diseased vessels. These data were collected prospectively and entered into a computerized database.

Statistical Analysis
Categorical variables were compared using the chi-
square test or the Fischer exact test. Continuous variables were compared using a t-test. Logarithm transformation of hs-CRP and sCD40L were used to improve the normality of these 2 inflammatory mediators substantially declined and hs-CRP were distinctively higher pre-procedure; both sCD40L and hs-CRP in Patients

Table 2 Serial Changes in Circulating Concentrations of sCD40L and hs-CRP in 77 Patients

<table>
<thead>
<tr>
<th></th>
<th>Pre-procedure</th>
<th>Day 21</th>
<th>Day 90</th>
<th>Day 180</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP (mg/L)</td>
<td>3.46±3.02a</td>
<td>1.51±1.16b</td>
<td>2.15±1.92b</td>
<td>1.92±1.83b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>With statin use</td>
<td>3.83±3.07a</td>
<td>1.71±1.38b</td>
<td>2.27±2.16b</td>
<td>2.02±1.92b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Without statin use</td>
<td>2.90±2.91a</td>
<td>1.22±0.66b</td>
<td>1.98±1.5b</td>
<td>1.78±1.70b</td>
<td>0.0007</td>
</tr>
<tr>
<td>With vs without statin use *</td>
<td>p=0.016</td>
<td>p=0.254a</td>
<td>p=0.498</td>
<td>p=0.379</td>
<td></td>
</tr>
<tr>
<td>sCD40L (pg/ml)</td>
<td>1.54±9.99a</td>
<td>532±470ab</td>
<td>762±733ab</td>
<td>789±606ab</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>With statin use</td>
<td>1.58±7.92a</td>
<td>523±414ab</td>
<td>864±790ab</td>
<td>784±590ab</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Without statin use</td>
<td>1.47±1.14a</td>
<td>547±550ab</td>
<td>749±606ab</td>
<td>793±625ab</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>With vs without statin use *</td>
<td>p=0.542</td>
<td>p=0.872</td>
<td>p=0.132</td>
<td>p=0.241</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Comparison of Baseline Characteristics Between Non-Restenosis and Restenosis Patients

<table>
<thead>
<tr>
<th></th>
<th>Non-restenosis (n=55)</th>
<th>Restenosis (n=22)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean±SD)</td>
<td>60.7±11.0</td>
<td>62.0±8.3</td>
<td>0.623</td>
</tr>
<tr>
<td>Male gender</td>
<td>83.6% (46)</td>
<td>68.2% (15)</td>
<td>0.212</td>
</tr>
<tr>
<td>Hypertension</td>
<td>56.4% (31)</td>
<td>68.2% (15)</td>
<td>0.339</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>23.6% (13)</td>
<td>45.5% (10)</td>
<td>0.059</td>
</tr>
<tr>
<td>Current smoking</td>
<td>47.3% (26)</td>
<td>36.4% (8)</td>
<td>0.384</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>54.6% (30)</td>
<td>40.9% (9)</td>
<td>0.280</td>
</tr>
<tr>
<td>Previous myocardic infarction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous stroke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)*</td>
<td>26.1±4.4</td>
<td>26.6±5.3</td>
<td>0.659</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.07±0.24</td>
<td>1.0±0.21</td>
<td>0.411</td>
</tr>
<tr>
<td>WBC count (&lt;10x10⁶/ml)</td>
<td>7.1±2.1</td>
<td>7.1±2.2</td>
<td>0.939</td>
</tr>
<tr>
<td>Statin use</td>
<td>60.0% (33)</td>
<td>59.1% (13)</td>
<td>0.941</td>
</tr>
</tbody>
</table>

Table 4 lists the relevant clinical outcomes and angiographic findings in restenotic and non-restenotic patients. No significant differences were noted in age, gender, coronary artery disease risk factors, previous MI or stroke, body mass index, laboratory findings, and statin use between the restenotic and non-restenotic patients (Table 3).

Table 4 lists the relevant clinical outcomes and angiographic findings in restenotic and non-restenotic patients. No significant differences were noted in age, gender, coronary artery disease risk factors, previous MI or stroke, body mass index, laboratory findings, and statin use between the restenotic and non-restenotic patients (Table 3).

**Results**

Comparison of Baseline Characteristics and Laboratory Findings Between Study Patients and Healthy Subjects

Table 1 summarizes the baseline characteristics of unstable angina patients and the healthy subjects. The 2 groups did not differ significantly in terms of age, gender, body mass index, and creatinine concentrations. However, the study patients had substantially higher serum concentrations of hs-CRP, sCD40L and white blood cell count than the normal control subjects.

Serial Changes in Circulating Concentrations of sCD40L and hs-CRP in Patients

In study patients, the serum concentrations of sCD40L and hs-CRP were distinctively higher pre-procedure; both of these 2 inflammatory mediators substantially declined after stenting. Meanwhile, the hs-CRP concentration did not vary significantly among the 3 intervals (on days 21, 90 and 180) in the patients; the sCD40L concentration declined markedly on day 21 and then rebounded (on days 90 and 180) to a significantly higher concentration (all p values <0.05). The sCD40L concentration did not differ between patients who did or did not receive statin therapy at the same interval. Additionally, the hs-CRP concentration also did not vary significantly among patients who did or did not receive statin therapy at the same interval after the procedure. However, the pre-procedure hs-CRP concentration was significantly higher in patients who did receive statin therapy compared to those who did not (Table 2).
cantly smaller, as was post-stented MLD in the restenotic group when compared to the non-restenotic group. Additionally, 6-month angiographic findings demonstrated that both MLD and RLD were substantially smaller in the restenotic group than in non-restenotic group.

Table 5 lists the serial changes in circulating concentrations of sCD40L and hs-CRP in patients with unstable angina undergoing elective coronary stenting, produced several striking clinical implications. First, serum concentrations of sCD40L and hs-CRP were markedly higher pre-procedure in study patients than in healthy subjects. Second, both these inflammatory mediators declined substantially after the procedure. However, the serum concentration of sCD40L, but not hs-CRP, remained significantly persistently higher in the study patients after the procedure when compared with healthy subjects. Third, a rebound phenomenon in the serum concentration of sCD40L was observed after cessation of clopidogrel therapy. Finally, the present study found no association between the serum concentration of these 2 inflammatory mediators and late restenosis.

Impact of Circulating Concentrations of sCD40L and hs-CRP on Plaque Instability

This investigation demonstrated that the circulating concentration of sCD40L was markedly elevated in patients with unstable angina who were undergoing stenting. This finding suggests that such a sharp increase in the serum concentration of sCD40L must be a fundamentally important prerequisite for developing unstable plaque, which propagates to rupture in the coronary artery. This suggestion, which is based on both clinical observations and laboratory findings, is supported by previous studies.

Previous studies have suggested that a raised serum concentration of hs-CRP may not only mirror an inflammatory

**Table 4** Comparison of Angiographic Findings Between Non-Restenosis and Restenosis in the Dilated Segment

<table>
<thead>
<tr>
<th></th>
<th>Non-restenosis (n=55)</th>
<th>Restenosis (n=22)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined 30-day MACE</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Six-month MI or death</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Multivessel disease</td>
<td>61.8% (34)</td>
<td>72.7% (16)</td>
<td>0.365</td>
</tr>
<tr>
<td>Pre-procedural findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion length (mm)</td>
<td>18.2±8.1</td>
<td>21.1±11.1</td>
<td>0.232</td>
</tr>
<tr>
<td>MLD (mm)</td>
<td>0.47±0.32</td>
<td>0.40±0.30</td>
<td>0.269</td>
</tr>
<tr>
<td>RLD (mm)</td>
<td>3.45±0.64</td>
<td>3.05±0.49</td>
<td>0.060</td>
</tr>
<tr>
<td>Post-stent findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLD (mm)</td>
<td>3.70±0.87</td>
<td>3.13±0.54</td>
<td>0.004</td>
</tr>
<tr>
<td>RLD (mm)</td>
<td>3.97±0.71</td>
<td>3.50±0.52</td>
<td>0.006</td>
</tr>
<tr>
<td>Six-month angiographic results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLD (mm)</td>
<td>2.47±0.67</td>
<td>0.72±0.58</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RLD (mm)</td>
<td>3.46±0.74</td>
<td>2.84±0.48</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are expressed as mean value ± SD or % (number) of patients.

MACE, major adverse cardiac event; MI, myocardial infarction; MLD, minimal lumen diameter; RLD, reference lumen diameter.

**Table 5** Comparison of Serial Changes in Serum Concentrations of hs-CRP and sCD40L Between Non-Restenosis and Restenotic Patients

<table>
<thead>
<tr>
<th></th>
<th>Pre-procedure</th>
<th>Day 21</th>
<th>Day 90</th>
<th>Day 180</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-restenosis (NR)†</td>
<td>3.57±3.08</td>
<td>1.50±1.10</td>
<td>2.10±1.94</td>
<td>1.95±1.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Restenosis (R)†</td>
<td>3.18±2.92</td>
<td>1.53±1.42</td>
<td>2.28±1.90</td>
<td>1.85±1.80</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>R vs NR*</td>
<td>p=0.799</td>
<td>p=0.630</td>
<td>p=0.481</td>
<td>p=0.949</td>
<td></td>
</tr>
<tr>
<td>sCD40L (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-restenosis (NR)†</td>
<td>1.48±0.50</td>
<td>0.49±0.50</td>
<td>0.75±0.74</td>
<td>0.81±0.60</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Restenosis (R)†</td>
<td>1.69±0.15</td>
<td>0.49±0.36</td>
<td>0.70±0.71</td>
<td>0.71±0.57</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>R vs NR*</td>
<td>p=0.310</td>
<td>p=0.618</td>
<td>p=0.196</td>
<td>p=0.235</td>
<td></td>
</tr>
<tr>
<td>Aspirin use</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Clopidogrel use</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean value ± SD.

sCD40L, soluble CD40 ligand; hs-CRP, high-sensitivity C-reactive protein.

†Compared at same time point; †Compared with normal subjects.

**Discussion**

The present study, which examined serum concentrations of sCD40L and hs-CRP in patients with unstable...
stimulus, but also directly promote atherosclerotic propagation and destabilization of plaque. The present study found substantially raised serum concentrations of hs-CRP in patients with unstable angina, implying that atherosclerotic plaque in a fissuring or ruptured situation was present, which further supports the previous suggestion that an elevated hs-CRP concentration may portend rupture of vulnerable plaque in patients with AMI or even cause sudden cardiac death.

**Time-Course of Circulating Concentrations of hs-CRP and sCD40L in Patients With Unstable Angina**

This investigation found that the serum concentration of sCD40L and hs-CRP declined substantially after stenting. This phenomenon could result from recovery from acute inflammation or from a successful procedure that reduces the progression of inflammation in the plaque lesion, or alternatively, may be a synergistic effect of physiological and pharmacological effects.

Interestingly, a marked decline in serum concentration on day 21, with a rebound on days 90 and 180, occurred with sCD40L, but not hs-CRP, for reasons that are still unknown. Previous studies have demonstrated that platelets are major contributors to enhanced concentrations of sCD40L in patients with unstable angina. Recently, we showed that clopidogrel suppresses platelet activation more substantially than aspirin in patients who had suffered an acute ischemic stroke. Additionally, only clopidogrel treatment was significantly independently associated with decreased CD62 expression on day 90. In the present study, clopidogrel was given to the patients for 4 weeks, then discontinued. With respect to previous findings, and taking our strategic management into consideration, it would, therefore, not be surprising to observe that the blood concentration of CD40L in the present patients declined on day 21 and then rebounded again on days 90 and 180.

A recent study showed that CRP upregulates the complement-inhibitory proteins that protect endothelial cells from complement-mediated cell injury. Therefore, a balance of the pro-atherogenic and anti-atherogenic effects of CRP on the vessel wall may be important in the development of atherosclerosis. Accordingly, we suggest that our findings may reflect inherently different pathways between sCD40L and hs-CRP for balancing the progression of inflammation and the anti-inflammatory response following an acute inflammatory stimulus. This hypothesis is based on differences in the cellular sources of these inflammatory biomarkers. It is well recognized that CRP, an acute inflammatory reactant, is primarily synthesized and secreted rapidly in the liver after an acute inflammatory stimulus. In contrast, sCD40L is derived from both CD4+ T cells and activated platelets.

The present study found a statistically significant and persistently elevated post-procedural serum concentration of sCD40L, compared with hs-CRP, in study patients compared with health subjects, suggesting that a raised sCD40L concentration is at least as useful as CRP for predicting future cardiovascular events in various clinical settings.

**Lack of Association Between Raised sCD40L and hs-CRP and Late Restenosis**

A link between raised inflammatory mediators and untoward short- and long-term outcomes has been well recognized in various clinical settings. Although an association has been found between inflammatory cytokines and restenosis after balloon angioplasty, the relationship between raised CRP and restenosis after percutaneous coronary intervention (PCI) remains inconsistent. Furthermore, the available data regarding the influence of these inflammatory mediators on restenosis after stenting is still limited and inconsistent. Importantly, the present study failed to find any association between raised CD40L and hs-CRP and late restenosis, which is consistent with recent studies. Some studies have failed to find any association between these inflammatory mediators and restenosis in patients undergoing either directional atherectomy or coronary artery stenting, suggesting that different treatment strategies may eliminate the ability of clinical factors or inflammatory biomarkers to predict late restenosis.

The mechanism of restenosis after PCI has been extensively debated. Although immediate elastic recoil, late constrictive geometric remodeling and neointimal hyperplasia constitute the main mechanisms of restenosis after balloon angioplasty; neointimal hyperplasia, is the essential mechanism of restenosis after stent implantation, particularly in diabetic patients. More recently, drug-eluting stents have emerged as a very attractive strategic management for preventing late restenosis in patients undergoing PCI. Inhibition of vascular smooth muscle cell proliferation by a drug-eluting stent is the principally mechanistic basis of how the restenotic rate is lowered. These clinical findings and our observations contribute to an understanding that different strategies may eliminate the ability of clinical factors or inflammatory biomarkers to act as influential predictors of late restenosis.

Notably, the present study found no association between inflammation and restenosis after stenting, which was supported by the results for the 2 inflammatory mediators. This prompts us to re-consider the role of the inflammation on stenosis if we choose a new treatment strategy. Another important finding of the present study was that post-PCI MLD was strongly associated with late restenosis after stenting, a finding that reinforces the results of an earlier study in which the final MLD and lesion length were found to be independently related to late restenosis.

**Study Limitations**

First, the sample size was relatively small, which may have distorted some important variables that only appeared to have a tendency of statistical significance. Second, because of the small sample size and the lack of major adverse cardiac events during the 6-month follow up, this investigation could not provide information regarding the relationship between the serum concentrations of sCD40L and hs-CRP and untoward clinical outcomes. Third, the sensitivity of the intravascular ultrasound may reinforce the claimed association between serum concentrations of sCD40L and hs-CRP and restenosis. Hence, the degree of sensitivity in the diagnosing restenosis may not be optimal. Finally, statins have been shown to have an anti-inflammatory effect and prevent atherosclerotic progression. Although serum concentrations of hs-CRP did not differ between therapy with and without statins in patients after stenting, the results of the present study demonstrated that the baseline concentration of serum hs-CRP and incidence of hypercholesterolemia were significantly higher in pa-
tients who were treated with statins than in patients who were not. Furthermore, the serum concentration of hs-CRP declined more substantially in patients who were treated with statins than in those patients who were not. Therefore, we still did not completely exclude the effect of this drug on lowering the serum concentration of hs-CRP, especially with such a low dose.

**Conclusion**

Circulating concentrations of sCD40L and hs-CRP were markedly increased during the acute phase in unstable angina patients, and declined substantially after coronary stenting. However, the circulating concentrations of these 2 inflammatory mediators were not good candidates for predicting late restenosis at the stent site.

**Acknowledgments**

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