Clinical Investigation and Reports

Soluble CD40L
Risk Prediction After Acute Coronary Syndromes

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Background—Elevated plasma concentrations of soluble CD40 ligand (sCD40L) indicate increased risk for future cardiovascular events in apparently healthy women. This study tested the hypothesis that plasma sCD40L, alone or in combination with troponin (cTnI) or C-reactive protein (CRP), may identify patients with acute coronary syndromes at heightened risk for recurrent cardiac events.

Methods and Results—In a nested case-control study (cases, n = 195; controls, n = 195) within the OPUS-TIMI16 trial, patients with the prespecified study end points death, myocardial infarction (MI), or congestive heart failure (CHF) within 10 months had significantly higher median (25th, 75th percentiles) sCD40L plasma levels than did controls (0.78 [0.34, 1.73] ng/mL versus 0.52 [0.16, 1.42] ng/mL, P < 0.002). After adjustment for other risk predictors and levels of cTnI and CRP, sCD40L levels above median were associated with higher risk for death, MI, and the composite death/MI or death/MI/CHF (adjusted hazard ratios, 1.9 [P < 0.05], 1.9 [P < 0.001], 1.9 [P < 0.001], and 1.8 [P < 0.01], respectively).

Interestingly, patients with elevated plasma levels of sCD40L and cTnI showed a markedly increased risk of death, MI, or death/MI/CHF compared with patients with the lowest levels of both markers (adjusted hazard ratios, 12.1, 7.2, and 4.3, respectively; all P < 0.01).

Conclusions—Elevated plasma levels of sCD40L identify patients with acute coronary syndromes at heightened risk of death and recurrent MI independent of other predictive variables, including cTnI and CRP. Notably, combined assessment of sCD40L with cTnI complements prognostic information for death and MI. (Circulation. 2003;108:1049-1052.)

Key Words: coronary disease ▪ myocardial infarction ▪ risk factors

CD40 signaling in endothelial and smooth muscle cells, monocytes, and platelets promotes a wide array of proatherogenic and prothrombotic functions in vitro and in vivo. In addition to the membrane-associated form, CD40L also occurs in plasma in soluble form (sCD40L). Elevated plasma concentrations of sCD40L at baseline foretell a significantly increased risk for future cardiovascular events in apparently healthy women. Furthermore, patients with unstable angina have higher plasma concentrations of sCD40L than healthy volunteers or those with stable angina, and elevation of sCD40L in this setting indicates a higher risk for recurrent events.

Elevated plasma levels of C-reactive protein (CRP) or troponin (cTnI) also predict increased future cardiovascular risk among apparently healthy subjects and patients with ACS. However, sCD40L plasma levels did not correlate with those of CRP or cTnI. The present study evaluated the relation between plasma sCD40L levels and outcomes in patients with acute coronary syndromes (ACS) and also tested the hypothesis that combined assessment of sCD40L and the validated markers cTnI or CRP enhances prediction.

Methods

Study Population
A nested case-control study was conducted among patients assigned to the placebo arm of the OPUS-TIMI16 trial who provided plasma samples at enrollment (within <72 hours of symptom onset). Cases were defined as death, myocardial infarction (MI), or new or progressive congestive heart failure (CHF) during a follow-up period of up to 10 months. For each case (n = 195), 1 control (n = 195) was matched based on age, gender, race, diabetes, smoking, index diagnosis, and performance of percutaneous coronary intervention as treatment for the index event.

sCD40L and CRP were determined in citrated plasma by ELISA (BenderMedSystems and ICN Pharmaceuticals, respectively.). cTnI was measured using the Access AccuTnI immunoassay (Beckman Coulter). All assays were performed in duplicates by investigators blinded to clinical end points and case-control status.

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Statistical Analysis
Means and proportions for baseline variables were compared between cases and controls using Student’s t test for continuous variables and the $\chi^2$ test for categorical variables. Plasma levels of CD40L are reported as the median and interquartile range. Association between sCD40L and baseline variables was determined by Pearson’s test for continuous variables and the Wilcoxon rank-sum test for categorical variables. Comparison of sCD40L between cases and controls and between subjects with and without other clinical end points used the Wilcoxon rank-sum test. Association between sCD40L quartiles and case status was evaluated using conditional logistic regression with adjustment for prior history of hypertension, hypercholesterolemia, coronary disease, heart failure, Killip class, ST depression $\geq 0.5$ mm, creatinine clearance, cTnI, and CRP. Cox proportional hazard models were used to evaluate other end points. Because matching was based on case definition, these models also included the matching variables listed above; all hazard ratios reported in the results section were adjusted for these variables. Comparisons between groups were made with the Wilcoxon rank-sum test.

Results
sCD40 Levels and Clinical Outcomes
As expected, cases of death, MI, or CHF within 10 months exhibited greater prevalence of hypertension, prior coronary artery disease (CAD) and CHF, and cTnI and CRP levels as well as lower creatinine clearance (Table 1). sCD40L levels above the median were associated with prior CAD and CRP levels (both $P<0.05$). In contrast, we observed no association with age, gender, hypertension, diabetes, cTnI, or creatinine clearance (Table 2). Cases showed elevated median (25th, 75th percentiles) plasma sCD40L compared with controls (0.78 [0.34, 1.73] ng/mL versus 0.52 [0.16, 1.42] ng/mL, $P<0.002$). Moreover, patients with MI or either composite (death/MI or death/MI/CHF) had significantly higher sCD40L levels at enrollment (Table 3).

Hazard ratios (adjusted for prior history of hypertension, hypercholesterolemia, coronary disease, heart failure, Killip class, ST depression $>0.5$ mm, creatinine clearance, cTnI, and CRP) for the study end point death/MI/CHF increased with rising quartiles of sCD40L levels. Patients with sCD40L levels in the third and fourth quartiles were more likely to have MI (hazard ratio, 2.0 [$P=0.05$] and 2.4 [$P=0.01$], respectively) or the composite death/MI (hazard ratio, 1.7 [$P=0.05$] and 2.2 [$P=0.005$], respectively) (Figure 1). Compared with the lowest quartiles, rates of death in the highest quartiles tended to increase, but these differences did not achieve statistical significance.

Finally, multivariate analysis using sCD40L as a continuous variable revealed that sCD40L levels remain associated with higher risk of death ($P=0.026$), MI ($P=0.011$), and the composites death/MI ($P=0.003$) and death/MI/CHF ($P=0.015$).

Predictive Value of sCD40L Combined With cTnI or CRP
To elucidate the potential gains in predictive value by the combined assessment of sCD40L with cTnI or CRP, we divided study participants based on concentrations of biomarkers above versus equal to or below the following thresholds: sCD40L, 0.52 ng/mL (median); cTnI, 0.06 ng/mL; and CRP, 15 mg/L. Patients with low concentrations of sCD40L/cTnI or sCD40L/CRP were assigned to a reference category with a
relative risk of 1. Importantly, sCD40L provided markedly increased hazard ratio for death or the composites (death/MI, death/MI/CHF) when assessed in combination with cTnI (Figure 2). Moreover, high sCD40L levels identified patients at risk for MI or death/MI not detected by CRP alone (Figure 2).

**Discussion**

In a cohort of patients with ACS, sCD40L plasma concentrations above the median associated independently with risk for recurrent MI or composite death/MI or death/MI/CHF within 10 months. CHF did not associate with sCD40L, indicating that the association between sCD40L and the composite of death and MI drove the association between sCD40L and the original end point of the OPUS-TIMI16 trial (the composite of death/MI/CHF). Previous in vitro and in vivo studies demonstrated a crucial role for CD40L in multiple stages of atherosclerosis. The present demonstration that elevated plasma sCD40L precedes recurrent MI additionally supports the hypothesis that CD40L plays a central role in the pathophysiology of ACS and independently validates a recent report. Interestingly, assessment of sCD40L in combination with cTnI, a validated marker of myocardial damage, markedly improved risk stratification in the present study. Furthermore, sCD40L provided better risk prediction than CRP in accord with recognition that CRP, an established inflammatory marker, only weakly predicts recurrent MI.

In summary, sCD40L independently predicts the composite of death/MI/CHF and particularly of recurrent MI in patients with ACS. Notably, simultaneous assessment of sCD40L and cTnI, the current standard for recurrent MI prediction, yields independent and complementary prognostic information, thus enabling more powerful prediction of adverse cardiac outcomes.

Interpretation of the results of this study, however, requires care because of the relatively small number of patients.
Future studies using larger cohorts will be needed to validate the clinical use of sCD40L independently or in combination with other markers in the prediction of cardiovascular events after ACS.

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References

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