Lack of Association of Soluble CD40 Ligand with the Presence of Acute Myocardial Infarction or Ischemic Stroke in the Emergency Department

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BACKGROUND: Soluble CD40 ligand (sCD40L) has been proposed as a new risk marker for cardiovascular diseases; however, its possible role as a diagnostic marker in the emergency department (ED) has not yet been investigated.

METHODS: We investigated sCD40L for the diagnosis of acute myocardial infarction or ischemic stroke in 1089 consecutive patients (525 males, 564 females; age, 17–98 years; median, 56 years) in an ED treating mainly adults with medical or neurologic emergencies. We used a research assay from Roche Diagnostics to measure sCD40L in heparinized plasma prepared from routinely drawn blood samples.

RESULTS: Intraassay and interassay CVs in our laboratory ranged from 1.6%–4.2% and from 4.4%–4.9%, respectively. A multiple linear regression analysis revealed sCD40L concentration to be significantly associated with C-reactive protein concentration (P = 0.012) and platelet count (P < 0.001). In addition, a subgroup analysis revealed a significant association between smoking and sCD40L concentration (P = 0.006). All other tested variables, including discharge diagnosis, age, sex, and other laboratory variables, showed no significant associations.

CONCLUSIONS: In adults presenting to the ED, sCD40L is not useful as a diagnostic marker for acute cardiac, cerebrovascular ischemic, or thromboembolic events.

Numerous reports have suggested soluble CD40 ligand (sCD40L) to be a promising biomarker of atherothrombotic risk (1–4). CD40L is a homotrimeric type II transmembrane protein that was originally identified in T lymphocytes, where it has a role in the immune response via binding to its receptor on B cells, CD40 (5). Both CD40 and CD40L have also been identified in cells within the vasculature (monocytes and macrophages), where they have been implicated as mediators of inflammation. Large amounts of CD40L are also located in intracellular vesicles of unstimulated platelets. The ligand becomes rapidly exposed on platelet surfaces after activation. Once exposed on the platelet surface, CD40L is proteolytically split into membrane-bound and soluble portions (sCD40L). sCD40L itself can activate unstimulated platelets. Despite the great research interest in this biomarker, the clinical use of sCD40L still awaits appropriate clinical validation, particularly as a diagnostic marker. Thus, the purpose of this study was to investigate plasma concentrations of sCD40L in consecutive patients in an emergency department (ED) to evaluate its utility for the diagnosis of acute myocardial infarction (AMI) or ischemic stroke in the acute setting.

This study was approved by the local ethics committee. We prospectively enrolled 1089 consecutive patients (ages, 17–98 years; 525 male, 564 women) who were admitted to the interdisciplinary ED of the University Hospital of Innsbruck from April 5 to April 29, 2006. This ED mainly treats internal medical and neurologic emergencies; pediatric, surgical, and traumatic emergencies are treated in separate EDs. Upon patient admission to the ED, blood samples were collected immediately into heparin-containing plastic tubes (Sarstedt) via a routinely placed intravenous cannula (Venflon®; BD Medical Systems) and centrifuged at 12 °C (2500g × 12 min) within 1 h of blood collection. CD40L was then measured in the remnants of these heparin-containing plasma samples within 2 h of blood collection with the Elecsys® sCD40L research test kit A2 (Roche Diagnostics), as has been previously described (6, 7). This test is an electrochemiluminescence sandwich immunoassay based on biotin–streptavidin technology. In our laboratory, intraassay and interassay CVs were 1.6%–4.2% and 4.4%–4.9%, respectively.

Patients were classified according to the discharge diagnosis, which was derived from careful chart review by experienced physicians blinded to sCD40L results.

5 Nonstandard abbreviations: sCD40L, soluble CD40 ligand; ED, emergency department; AMI, acute myocardial infarction; STEMI, ST-segment elevation myocardial infarction.
Patients with myocardial infarction were divided into ST-segment elevation myocardial infarction (STEMI) and non-STEMI groups according to the universal definition of myocardial infarction (8) (i.e., a rising and/or falling pattern of cardiac troponin T with at least one value above the 99th percentile in the context of typical symptoms and/or electrocardiographic changes indicative of myocardial ischemia). A second analysis considered patients with atherosclerotic or thromboembolic events (non-STEMI, STEMI, ischemic stroke, pulmonary embolism, venous thrombosis), because the pathophysiology of these conditions predicted that sCD40L concentrations would be increased in such patients. The control group included all remaining patients in each analysis.

sCD40L and other variables with nonnormal distributions were logarithmically transformed before statistical analysis. We calculated Pearson correlation coefficients and used ANOVA and Student t-tests to analyze sCD40L concentrations in the different disease groups. We used the Bonferroni adjustment to correct P values and used multiple linear regression analysis to investigate significant associations with sCD40L. We used a logistic regression model to evaluate the association of possible risk markers with the presence of acute ischemic or thromboembolic events (AMI, ischemic stroke, pulmonary embolism, or venous thrombosis). The SPSS software package (V12.01; SPSS) was used for all statistical analyses. P values <0.05 or Bonferroni-corrected P values were considered to indicate statistical significance.

Fig. 1 shows the distribution of sCD40L concentrations in the study population according to disease. sCD40L concentrations in the various disease groups were not significantly different (ANOVA, P > 0.05 after Bonferroni correction). sCD40L concentrations of men and women were also not significantly different (P = 0.497). The median sCD40L concentration was 0.187 μg/L in men and 0.189 μg/L in women. Age was not significantly associated with sCD40L concentration (r = 0.034; P = 0.262). A multiple linear regression analysis revealed higher C-reactive protein concentrations and higher platelet counts to be significant predictors of higher sCD40L concentrations (Table 1).
Table 1. Associations with sCD40L concentrations in multiple linear regression analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>P</th>
<th>Standardized β coefficient</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.467</td>
<td>0.030</td>
<td>0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>0.513</td>
<td>0.027</td>
<td>0.022</td>
</tr>
<tr>
<td>Ischemic event</td>
<td>0.686</td>
<td>0.016</td>
<td>0.047</td>
</tr>
<tr>
<td>C-reactive protein (log*</td>
<td>0.012b</td>
<td>0.146</td>
<td>0.024</td>
</tr>
<tr>
<td>Fibrinogen (log)</td>
<td>0.313</td>
<td>-0.060</td>
<td>0.098</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>0.440</td>
<td>0.033</td>
<td>0.003</td>
</tr>
<tr>
<td>Platelets</td>
<td>&lt;0.001b</td>
<td>0.190</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatine kinase (log)</td>
<td>0.196</td>
<td>0.052</td>
<td>0.034</td>
</tr>
</tbody>
</table>

* Log, log-transformed data.
*b Statistically significant association.

In a subgroup analysis of patients who had a well-documented smoking status and complete data (n = 73) revealed that smoking was significantly associated with higher sCD40L concentrations (P = 0.006).

sCD40L concentrations in patients who had been treated with drugs affecting coagulation (acetylsalicylic acid, n = 250; heparin, n = 26; clopidogrel, n = 39; coumarins, n = 55) before blood sampling were not significantly different from those who did not receive such drug treatment; however, patients who had been treated with heparin before blood sampling had significantly higher sCD40L concentrations than the other patients (P = 0.004).

We also used a logistic regression model to analyze risk markers for association with an acute ischemic or thromboembolic event (AMI, ischemic stroke, pulmonary embolism, or venous thrombosis). Age (odds ratio, 1.04/year; P < 0.001), known coronary artery disease (odds ratio, 6.71; P < 0.001), and a higher erythrocyte count (odds ratio, 2.14; P = 0.022) were identified as significant risk markers (see Table 1 in the Data Supplement that accompanies the online version of this Brief Communication at http://www.clinchem.org/content/vol55/issue1). In an additional subgroup analysis, we used a logistic regression model to investigate laboratory predictors of AMI in 157 patients presenting with acute chest pain. As expected, an increased troponin concentration was a highly significant biochemical marker for predicting AMI; however, its predictive value was not improved by adding other laboratory variables, including sCD40L.

Despite evidence for sCD40L as a useful marker for risk stratification (1–4), its utility as a diagnostic marker has not been sufficiently investigated. In this study, we were unable to demonstrate a clinical utility of sCD40L as an additional diagnostic marker for cardiovascular diseases in the ED setting; however, our results add to other published studies of negative results regarding the prognostic value of sCD40L. For example, Olenchock et al. (7) reported an absence of an association between sCD40L and cardiovascular outcomes in a large cohort of patients with acute coronary syndrome. In our ED study population, sCD40L concentrations did not differ significantly between groups with respect to sex, age, or disease. The absence of an association with age or sex is also in accordance with the results of Olenchock et al. (7). When comparing sCD40L results, it is very important to be aware of differences in sample type and the assays used. Preanalytical conditions, not always precisely reported in clinical studies, are critical for assessing sCD40L concentrations and study results, and several recent studies have demonstrated that plasma samples are preferred for sCD40L measurement, because sCD40L is released from platelets ex vivo during sample clotting (6, 7, 9–12). Our group and Olenchock et al. (7) measured sCD40L in plasma samples with the same automated immunoassay (Roche Diagnostics), which has been well validated analytically. Our AMI patients did not have significantly higher sCD40L concentrations, and even when we pooled all atherosclerotic or thromboembolic diseases, sCD40L concentrations were not significantly different from those in patients with other diseases. These results confirm the study of Tanne et al. (13), who found no association between high sCD40L serum concentrations and an increased risk for ischemic stroke or coronary events in patients with chronic coronary artery disease. Subsequent studies likewise have not detected any association between this biomarker and subsequent cardiovascular events (6). A multiple linear regression analysis revealed sCD40L concentration to be significantly associated with C-reactive protein concentration and platelet count. On a pathophysiological basis, the association of sCD40L with C-reactive protein is expected. Unexpectedly, however, patients with a diagnosis of acute infection did not show significantly higher sCD40L concentrations.

Our finding of a significant association between sCD40L and platelet count and the fact that large amounts of sCD40L in the circulation are derived from platelets (14) indicate that sCD40L can be regarded as a marker of platelet activation. In a subgroup analysis, we also found a significant effect of smoking on sCD40L concentration, confirming the reports of Harding et al., who demonstrated up-regulation of the CD40/CD40L dyad and platelet–monocyte aggregation in cigarette smokers (15), and Olenchock et al., who also reported a statistically significant association between sCD40L and smoking (7). Of all the labora-
tory variables we tested, troponin, as expected, was the most important biochemical marker for predicting AML. sCD40L did not have any additional independent predictive value in this logistic regression model. Of all the drugs we tested, only heparin therapy before blood sampling was associated with significantly different (higher) sCD40L concentrations. This finding is in accordance with the results of Keating et al., who found increased production of P-selectin in platelets from patients treated with unfractionated heparin plus eptifibatide, compared with bivalirudin (16).

In conclusion, in a “real world” adult ED population, sCD40L provided no additive value as a diagnostic test for acute cardiac or cerebrovascular ischemic events. This lack of diagnostic value might be explained by the high prevalence of diseases with procoagulant activity and/or inflammation in an ED population. Our results contribute to the growing awareness that sCD40L is a biomarker of very limited clinical utility in a routine setting.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

References


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