Serum Soluble Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 Levels Are Elevated in Acute Coronary Syndrome
A Novel Marker for Early Diagnosis

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Background—Markers of cardiac injury, including troponin-T (TnT), are used to diagnose acute coronary syndrome (ACS); however, markers for plaque instability may be more useful for diagnosing ACS at the earliest stage. Lectin-like oxidized LDL receptor-1 (LOX-1) appears to play crucial roles in the pathogenesis of atherosclerotic plaque rupture and ACS onset. LOX-1 is released in part as soluble LOX-1 (sLOX-1) by proteolytic cleavage.

Methods and Results—We examined serum sLOX-1 levels in 521 patients, consisting of 427 consecutive patients undergoing coronary angiography, including 80 ACS patients, 173 symptomatic coronary heart disease patients, 122 patients with significant coronary stenosis without ischemia, and 52 patients without apparent coronary atherosclerosis plus 34 patients with noncardiac acute illness and 60 patients with noncardiac chronic illness. Time-dependent changes in sLOX-1 and TnT levels were analyzed in an additional 40 ACS patients. Serum sLOX-1 levels were significantly higher in ACS than the other groups and were associated with ACS as shown by multivariable logistic regression analyses. Given a cutoff value of 1.0 ng/mL, sLOX-1 can discriminate ACS from other groups with 81% and 75% of sensitivity and specificity, respectively. sLOX-1 can also discriminate ACS without ST elevation or abnormal Q waves and ACS without TnT elevation from non-ACS with 91% and 83% of sensitivity, respectively. Peak values of sLOX-1 in ACS were observed earlier than those of TnT.

Conclusions—sLOX-1 appears to be a useful marker for early diagnosis of ACS. (Circulation. 2005;112:812-818.)

Key Words: angina ■ atherosclerosis ■ lipoproteins ■ myocardial infarction ■ receptors

Acute coronary syndrome (ACS) is one of the major causes of mortality and morbidity in developed countries. Accurate diagnosis of ACS at the earliest stage would improve prognosis through appropriate treatment without delay. ACS appears to be provoked by a rupture of lipid-rich atheromatous plaques, followed by thrombus formation. Several diagnostic tests such as echocardiography, radioisotope scintigraphy, and measurement of circulating levels of troponin-T (TnT) and the MB isofrom of creatine kinase have been used to detect ischemic myocardial damage in clinical practice; however, none of these markers directly indicates plaque instability or rupture before myocardial damage becomes apparent. Such markers for plaque instability or rupture would establish the diagnosis of ACS at the earliest stage and may predict the onset. Several serum markers, including high-sensitivity C-reactive protein (hs-CRP), oxidized LDL (Ox-LDL), and soluble forms of membrane proteins such as CD40 ligand (CD40L), ICAM-1, and E-selectin were reported to be associated with ACS or acute myocardial infarction. Although soluble CD40L has recently been shown to be correlated with prognosis after ACS, none of these markers has been established as a diagnostic marker of ACS at the earliest stage.

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LDL-lowering therapy has been shown to decrease the incidence of ACS and other atherosclerosis-related diseases.
es.15–18 In addition, the importance of oxidatively modified LDL has been demonstrated in this process.19,20 In fact, plasma Ox-LDL levels have been shown to be elevated in patients with ACS.9 Effects of Ox-LDL on vascular cells in atherosclerotic progression and plaque rupture appear to be mediated by its receptors.21 Lectin-like oxidized LDL receptor-1 (LOX-1) is a receptor with an expression that is not constitutive but dynamically inducible by proinflamma-
tory stimuli, angiotensin II, and Ox-LDL, which are risk factors for ACS.22–28 In human atherosclerotic lesions, LOX-1 is expressed prominently by intimal smooth muscle cells and lipid-laden macrophages in the advanced plaques.29 Furthermore, LOX-1 plays an important role in Ox-LDL–
induced apoptosis of vascular smooth muscle cells30,31 and production of matrix metalloproteinases,32 which may di-
rectly be linked to plaque rupture. LOX-1 is also expressed on the surface of activated platelets,33 which may also be involved in thrombus formation after plaque rupture.

LOX-1 expressed on the cell surface can be proteolytically cleaved at its membrane proximal extracellular domain and released as soluble forms (sLOX-1).34 Therefore, we have established a specific and sensitive assay to measure concentra-
tions of sLOX-1 in human sera. The present report shows that serum sLOX-1 levels are elevated in ACS from its early stage, suggesting its usefulness as an early diagnostic marker of ACS.

Methods

Patient Sample

We enrolled 427 patients who underwent diagnostic coronary an-
goingy (CAG) at the cardiovascular center and 34 patients who visited the emergency department and immediately were hospitalized in the Osaka Red Cross Hospital because of severe noncardiac acute diseases such as infectious diseases, trauma, and asthmatic fit and 60 patients with chronic problems in the outpatient department of internal medicine. All subjects were consecutively identified. All patients in this study gave written informed consent. Consecutive patients undergoing CAG were assigned to 4 groups depending on CAG findings and clinical features. Fifty-two patients whose CAG did not show any apparent atherosclerotic lesions were assigned to the group of patients with intact coronary. One hundred twenty-two patients who had documented coronary atherosclerosis by CAG but had been free of episodes of angina or documented cardiac ischemia for at least 3 months were assigned to the group of patients with controlled coronary heart disease (CHD). One hundred seventy-three patients who had significant coronary stenosis and ischemic symp-
toms (stable angina) and required elective coronary artery revascular-
ization procedures such as percutaneous coronary intervention (PCI) or CABG were assigned to the group of patients with ischemic CHD. Eighty patients presented with ACS, which was defined as acute onset of prolonged chest pain or discomfort accompanied by ST-segment elevation or depression evolving into pathological Q waves or T-wave inversion and emergency CAG-documented total occlusion or marked delayed filling of a coronary artery. Among ACS patients, those without ST-segment elevation or pathological Q waves were defined as non–Q-wave ACS (NQ-ACS).

In another group of 40 ACS patients, serum sLOX-1 and TnT were serially measured on admission (at 4.4 ± 4.2 hours after onset), immediately after emergency PCI, and at days 1, 3, 5, and 7. Patients with symptomatic peripheral vascular diseases were excluded from this study.

This study, carried out in accordance with the principles of the Declaration of Helsinki, was approved by local ethics committees.

Measurement of sLOX-1 and Other Serum Markers

Serum samples were collected at coronary angiography for patients undergoing CAG or at time of visit for patients with acute illness and chronic illness. In a time-dependent analysis, serum samples were collected serially at the indicated time periods. These samples were stored at −80°C until assays were performed. Serum sLOX-1 levels were determined by a sandwich ELISA using 2 different human LOX-1–specific antibodies. Antibodies were obtained after purification of serum from 2 different rabbits that had been immunized with a recombinant protein corresponding to the extracellular domain of human LOX-1. One of these antibodies was used to coat the plates; the other was fragmented into Fab’ and labeled with horseradish peroxidase for enzymatic detection. Standard curves were obtained by use of a recombinant protein corresponding to the extracellular domain of human LOX-1. Intra-assay and interassay coefficients of variation were 2.0% to 11.8% and 0.0% to 8.1%, respectively. The lower limit of the detection for sLOX-1 was 0.5 ng/mL. All assays were carried out by personnel who had no knowledge of the clinical diagnosis of the patients. Measurement of diluted serum samples by the same ELISA (see the Figure in the online-only Data Supplement) and immunoprecipitation followed by immunoblotting (data not shown) showed comparable results, indicating the accuracy and reliability of this ELISA for sLOX-1. Levels of hs-CRP and TnT were determined on the same serum samples as those for sLOX-1 by commercially available electrochemiluminescent immunoassay kit (F. Hoffmann–La Roche Ltd, and particle-enhanced immunoneph-
elometry (Dade Behring Ltd), respectively.

Statistical Analysis

We performed statistical analysis using Stat-View, version 5, and SPSS. The 1-way ANOVA was used to compare clinical continuous variables with the Tukey–Kramer test for multiple comparisons and 2-way cross-tabulation with the χ2 test for binary variables, when appropriate, to compare differences between groups. When sLOX-1 was undetectable by ELISA, the sLOX-1 level was assigned 0. Levels of sLOX-1 did not distribute normally; therefore, the Kruskal-Wallis and Dunn’s tests were used for multiple compari-
sions. Association between sLOX-1 and hs-CRP, LDL cholesterol, HDL cholesterol, triglycerides, or TnT was evaluated by Spearman’s rank correlation coefficient. Multivariable logistic regression analy-
sis was performed to assess the correlation between ACS and age, gender, hypertension, diabetes, smoking, LDL cholesterol, HDL cholesterol, triglycerides, hs-CRP, or sLOX-1.35 Transformed values of hs-CRP in logarithm were used as variables for statistical analyses. Time profiles of serum sLOX-1 and TnT levels were analyzed after conversion of the individual’s serial sLOX-1 levels into relative ratios to each individual’s maximum value by 1-way repeated-measures ANOVA and multiple comparisons with Bonferroni’s test. Receiver-operating characteristic (ROC) analysis was also carried out on the levels of sLOX-1 and hs-CRP for ACS and ACS without apparent ST elevation or pathological Q waves (NQ-ACS) separately. This analysis plots the true-positive fraction (sensitivity) against the false-positive fraction (1– specificity) by changing the cutoff value for the test. Areas under the ROC curves indicate the relative accuracy of diagnostic tests.36 All probability values are 2 sided. Values of P < 0.05 were considered statistically significant.

Results

Clinical Characteristics of the Study Samples

Table 1 summarizes age, gender, conventional cardiovascular risk factors, and lipid profiles in each group of patients, as well as the combined non-ACS patients, undergoing CAG. Patient characteristics, including age, gender, and incidence of hypertension, diabetes, and hypercholesterolemia, were comparable between the ACS group and the combined non-ACS CAG, except that the
TABLE 1. Characteristics of Consecutive CAG Patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Intact Coronary</th>
<th>Controlled CHD</th>
<th>Ischemic CHD</th>
<th>Non-ACS CAG</th>
<th>ACS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>52</td>
<td>122</td>
<td>173</td>
<td>Subtotal, 347</td>
<td>80</td>
</tr>
<tr>
<td>Age (mean±SD), y</td>
<td>66±9</td>
<td>66±10</td>
<td>67±9</td>
<td>67±10</td>
<td>64±12</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>32 (62)</td>
<td>89 (73)</td>
<td>128 (74)</td>
<td>249 (67)</td>
<td>59 (74)</td>
</tr>
<tr>
<td>Risk factors, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>22 (42)</td>
<td>65 (53)</td>
<td>82 (47)</td>
<td>168 (48)</td>
<td>30 (38)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>8 (15)†</td>
<td>43 (35)</td>
<td>63 (36)</td>
<td>114 (33)</td>
<td>26 (33)</td>
</tr>
<tr>
<td>Smoking</td>
<td>19 (37)</td>
<td>61 (50)</td>
<td>57 (33)¶</td>
<td>136 (39)*</td>
<td>43 (54)*</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>14 (27)</td>
<td>47 (39)</td>
<td>84 (49)$</td>
<td>144 (41)</td>
<td>27 (34)</td>
</tr>
</tbody>
</table>

Lipid profile (mean±SD), mg/dL

<table>
<thead>
<tr>
<th></th>
<th>LDL cholesterol</th>
<th>HDL cholesterol</th>
<th>Triglycerides</th>
<th>hs-CRP (mean±SD), ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact Coronary</td>
<td>122±38</td>
<td>125±35</td>
<td>121±35</td>
<td>3.10±0.75</td>
</tr>
<tr>
<td>Controlled CHD</td>
<td>125±35</td>
<td>121±35</td>
<td>121±36</td>
<td>3.09±0.65</td>
</tr>
<tr>
<td>Ischemic CHD</td>
<td>121±35</td>
<td>121±36</td>
<td>121±36</td>
<td>3.11±0.87</td>
</tr>
<tr>
<td>Non-ACS CAG</td>
<td>122±35</td>
<td>122±36</td>
<td>122±36</td>
<td>3.10±0.78¶</td>
</tr>
<tr>
<td>ACS</td>
<td>122±35</td>
<td>122±36</td>
<td>122±35</td>
<td>3.41±0.87¶#</td>
</tr>
</tbody>
</table>

Values for hs-CRP were transformed in logarithm of 10. One-way ANOVA was followed up with Tukey-Kramer pairwise comparisons among means.

*P<0.01 for comparison with combined all non-ACS and ACS with 2-way cross-tabulation with χ² test.
†P<0.05 for comparison with controlled CHD, ischemic CHD, and ACS.
‡P<0.05 for comparison with controlled CHD and ACS.
§P<0.05 for comparison with intact coronary and ACS.
¶P<0.05 for comparison with combined all non-ACS and ACS with 2-way cross-tabulation with χ² test.
*P<0.05 for comparison with intact coronary.
†P<0.05 for comparison with controlled CHD.
‡P<0.05 for comparison with ischemic CHD.
§P<0.05 for comparison with non-ACS CAG and ACS with t test.
¶P<0.05 for comparison with acute illness.
#P<0.05 for comparison with chronic illness.
ACS group showed higher smoking rate and lower HDL cholesterol levels (Table 1). Table 2 compares the patient characteristics among ACS, non-ACS CAG, and noncardiac acute and chronic illness groups. Patient characteristics were comparable between the ACS and combined non-ACS group, except that HDL cholesterol levels were significantly lower and the incidence of smoking habits was significantly higher in ACS than in the combined all non-ACS group (Table 2), as shown in CAG groups alone (Table 1).

TABLE 2. Characteristics of All Enrolled Patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Noncardiac Chronic Illness</th>
<th>Noncardiac Acute Illness</th>
<th>Non-ACS CAG</th>
<th>Combined All Non-ACS</th>
<th>ACS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>60</td>
<td>34</td>
<td>347</td>
<td>Subtotal, 441</td>
<td>80</td>
</tr>
<tr>
<td>Age (mean±SD), y</td>
<td>67±16</td>
<td>54±18†</td>
<td>67±10</td>
<td>66±13</td>
<td>64±12</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>18 (30)¶</td>
<td>21 (62)</td>
<td>249 (67)</td>
<td>288 (65)</td>
<td>59 (74)</td>
</tr>
<tr>
<td>Risk factors, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>16 (27)</td>
<td>9 (26)</td>
<td>168 (48)$</td>
<td>193 (44)</td>
<td>30 (38)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>5 (8)$§</td>
<td>2 (6)$§</td>
<td>114 (33)</td>
<td>121 (27)</td>
<td>26 (33)</td>
</tr>
<tr>
<td>Smoking</td>
<td>9 (15)¶</td>
<td>13 (38)</td>
<td>136 (39)</td>
<td>158 (36)*</td>
<td>43 (54)*</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>25 (42)</td>
<td>6 (18)†</td>
<td>144 (41)</td>
<td>175 (40)</td>
<td>27 (34)</td>
</tr>
</tbody>
</table>

Lipid profile (mean±SD), mg/dL

<table>
<thead>
<tr>
<th></th>
<th>LDL cholesterol</th>
<th>HDL cholesterol</th>
<th>Triglycerides</th>
<th>hs-CRP (mean±SD), ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncardiac Chronic Illness</td>
<td>127±30</td>
<td>101±37†</td>
<td>121±36</td>
<td>3.14±0.58</td>
</tr>
<tr>
<td>Noncardiac Acute Illness</td>
<td>121±36</td>
<td>122±36</td>
<td>122±36</td>
<td>3.10±0.78</td>
</tr>
<tr>
<td>Non-ACS CAG</td>
<td>122±35</td>
<td>122±35</td>
<td>122±35</td>
<td>3.41±0.87#</td>
</tr>
</tbody>
</table>

Values for hs-CRP were transformed in logarithm of 10. One-way ANOVA was followed up with Tukey-Kramer pairwise comparisons among means.

*P<0.01 for comparison with combined all non-ACS and ACS with 2-way cross-tabulation with χ² test.
†P<0.05 for comparison with chronic illness, non-ACS CAG, and ACS.
‡P<0.05 for comparison with acute illness, chronic illness, and non-ACS CAG.
§P<0.05 for comparison with non-ACS CAG and ACS.
¶P<0.05 for comparison with combined all non-ACS and ACS with 2-way cross-tabulation with χ² test.
*P<0.05 for comparison with chronic illness.
†P<0.05 for comparison with non-ACS CAG and ACS.
‡P<0.05 for comparison with acute illness.
§P<0.05 for comparison with non-ACS CAG.
¶P<0.05 for comparison with chronic illness.
#P<0.05 for comparison with non-ACS CAG.
Factors, hs-CRP, and sLOX-1

Serum sLOX-1 Levels

As shown in Figure 1A, serum sLOX-1 levels were remarkably higher in ACS (median, 2.91 ng/mL; range, <0.5 to 170 ng/mL) when compared among 6 groups including intact coronary (median, <0.5 ng/mL; range, <0.5 to 1.3 ng/mL), controlled CHD (median, <0.5 ng/mL; range, <0.5 to 3.4 ng/mL), ischemic CHD (median, 0.73 ng/mL; range, <0.5 to 14.0 ng/mL), acute noncardiac illness (median, <0.5 ng/mL; range, <0.5 to 6.4 ng/mL), and chronic illness (median, <0.5 ng/mL; range, <0.5 to 3.3 ng/mL). Serum sLOX-1 can discriminate ACS from other CAG groups (χ²=88.2, P<0.001), given a cutoff value of 1.0 ng/mL, with 81% sensitivity and 75% specificity (Table 3).

Lipid Profiles, Conventional Cardiovascular Risk Factors, hs-CRP, and sLOX-1

Serum hs-CRP levels were significantly higher in the ACS than non-ACS groups when compared among 4 CAG groups alone (Table 1 and Figure 1B). Levels of hs-CRP in patients with noncardiac acute illness were significantly higher than in any of other groups because this group contained acute inflammatory diseases (Figure 1B and Table 2). Although levels of hs-CRP in patients with ACS were significantly higher than in any of other groups when compared among CAG patients alone, ACS did not show statistically significant difference in serum hs-CRP levels when compared among all the 6 groups, including noncardiac acute and chronic illness groups (Figure 1B and Table 2).

Significant inverse correlation was found between sLOX-1 and HDL cholesterol levels (Spearman’s ρ = -0.17; P<0.01). However, no significant correlation was found between sLOX-1 and either LDL cholesterol (Spearman’s ρ = -0.02; P=0.68) or triglyceride (Spearman’s ρ = -0.01, P=0.89) levels. We also examined the association between sLOX-1 levels and other cardiovascular risk factors such as hypertension, diabetes, and smoking among all enrolled patients. No significant differences were found in sLOX-1 levels between those with and without hypertension, diabetes, or smoking.

Multivariable logistic regression analyses of all patients (Cox and Snell’s R²=0.263) showed that sLOX-1 was associated with ACS (odds ratio, 1.51; 95% CI, 1.35 to 1.70; P<0.001). Levels of hs-CRP, HDL cholesterol, and smoking habits also were significantly associated with ACS (odds ratio, 1.40, 0.96, and 2.07; 95% CI, 1.00 to 1.94, 0.94 to 0.98, and 1.08 to 3.96; P<0.05, P<0.01, and P<0.05, respectively). However, no significant correlation was found between sLOX-1 and hs-CRP levels among all patients and patients with ACS alone (Spearman’s ρ=0.01 and −0.06; P=0.81 and P=0.58, respectively).

sLOX-1 as a Diagnostic Marker of ACS

Figure 2 shows ROC curves for the levels of sLOX-1 and hs-CRP in all 80 ACS patients (Figure 2A) and 24 patients with ACS without ST elevation or abnormal Q waves at the time of visit (NQ-ACS) (Figure 2B) compared with the 347

Table 3. Sensitivity and Specificity of sLOX-1 and hs-CRP for ACS Among CAG Patients

<table>
<thead>
<tr>
<th></th>
<th>sLOX-1</th>
<th>hs-CRP</th>
<th>TnT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive, n</td>
<td>86</td>
<td>91</td>
<td>...</td>
</tr>
<tr>
<td>Specificity</td>
<td>75</td>
<td>74</td>
<td>...</td>
</tr>
<tr>
<td>All ACS (n=80)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive, n</td>
<td>65</td>
<td>36</td>
<td>54</td>
</tr>
<tr>
<td>χ²</td>
<td>88.2</td>
<td>12</td>
<td>...</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>...</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>81</td>
<td>45</td>
<td>68</td>
</tr>
<tr>
<td>NQ-ACS (n=23)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive, n</td>
<td>21</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>χ²</td>
<td>43.2</td>
<td>1.7</td>
<td>...</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>0.2</td>
<td>...</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>91</td>
<td>39</td>
<td>48</td>
</tr>
</tbody>
</table>

Cutoff values were 1.0 ng/mL for sLOX-1, 4 µg/mL for hs-CRP, and 0.03 ng/mL for TnT. ACS patients with <0.03 ng/mL TnT determined at the time of visit were defined as cases with TnT negative at the time of visit. χ² was determined by the Yates continuity-corrected χ² test, and probability values were obtained by comparison with non-ACS patients.
non-ACS CAG patients as a reference group. In all ACS patients, the areas below the curves were 0.86 (95% CI, 0.81 to 0.90) for sLOX-1 and 0.62 (95% CI, 0.55 to 0.69) for hs-CRP. In patients with NQ-ACS, the areas below the curves were 0.90 (95% CI, 0.86 to 0.94) for sLOX-1 and 0.63 (95% CI, 0.52 to 0.74) for hs-CRP. These differences between sLOX-1 and hs-CRP (0.24 and 0.27; 95% CI, 0.20 to 0.28 and 0.21 to 0.33, respectively) are statistically significant \((p < 0.05)\) in both all ACS and NQ-ACS patients. Given a cutoff value of 1.0 ng/mL for sLOX-1, serum sLOX-1 can significantly discriminate ACS patients from non-ACS patients (non-ACS CAG) among consecutive patients undergoing coronary angiography \((p < 0.001)\) and showed 81% sensitivity and 75% specificity for the diagnosis of ACS (Table 3). In contrast, an hs-CRP cutoff value of 4 \(\mu\)g/mL, which had comparable specificity (74%), showed lower sensitivity (45%) for the diagnosis of ACS. Values of sLOX-1 at the time of visit efficiently discriminated patients with NQ-ACS \((p < 0.001)\) from non-ACS CAG with 91% sensitivity; however, sensitivity of TnT (cutoff value, 0.03 ng/mL) for diagnosis of NQ-ACS was 48%. Moreover, sLOX-1 showed 83% sensitivity for diagnosis of ACS even in patients with negative TnT \(< 0.03 \text{ ng/mL}\) at the time of visit (Table 3).

### Time-Dependent Changes in sLOX-1 Concentrations After the Onset of ACS

Serum sLOX-1 and TnT were serially measured in consecutive 40 ACS patients. Figure 3A indicates relative values of serum sLOX-1 and TnT compared with the highest values among serial blood samples obtained from each individual patient. Peak levels of sLOX-1 were observed on admission or after PCI \((p < 0.01)\). In contrast, the highest TnT values were observed around day 1, which is consistent with previous reports \((p < 0.01)\). In addition, no significant correlation was found between peak levels of sLOX-1 and CPK (Spearman’s \(\rho = 0.28\); \(p = 0.10\)) or TnT (Spearman’s \(\rho = 0.20\); \(p = 0.20\); Figure 3B).

### Discussion

Rupture of atheromatous plaques, followed by thrombus formation, is considered a crucial step in the pathogenesis of ACS. Atherosclerotic plaques with abundant lipid-laden macrophages and activated smooth muscle cells in the intima appear to be prone to rupture.\(^{39}\) In such vulnerable plaques, LOX-1 is expressed prominently by smooth muscle cells and macrophages and contributes to apoptosis of smooth muscle cells\(^{29–31}\) and production of matrix metalloproteinases.\(^{32}\) Under these conditions, enhanced protease activities may cleave

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**Figure 2.** ROC curves of sLOX-1 and hs-CRP for diagnosis of ACS (A) and ACS without ST elevation or abnormal Q-waves (NQ-ACS; B) among consecutive patients undergoing coronary angiography. True-positive fraction (sensitivity as y axis) was plotted vs false-positive fraction (1—specificity as x axis) by changing cutoff values for test.

**Figure 3.** Time-dependent changes in sLOX-1 and TnT levels after onset of ACS (A) and comparison between peak values of sLOX-1 and TnT (B). Blood samples were collected on admission, immediately after PCI (post-PCI), and on days 1, 3, 5, and 7 from 40 ACS patients undergoing emergency PCI. Relative ratios (mean±SEM) to peak value of each individual patient are indicated (●, sLOX-1; ○, TnT). Statistically significant correlation was not found between peak values of sLOX-1 and TnT during these periods (Spearman’s \(\rho = 0.20\); \(p = 0.20\)).
sLOX-1 from the surface of these vascular cells in which LOX-1 is abundantly expressed, although proteases responsible for LOX-1 cleavage have not been fully identified. Additionally, in the process of thrombus formation after plaque rupture, LOX-1 expression on the surface of platelets may also be abundant by thrombotic activation, as is the case for CD40L. However, LOX-1 can also bind activated platelets; therefore, sLOX-1 might not be liberated from the surface of activated platelets. In fact, we did not observe significant differences in sLOX-1 levels between plasma and serum samples or high levels of circulating sLOX-1 in typical patients with disseminated intravascular coagulation (data not shown). Moreover, LOX-1 expression can be inducible in cardiac myocytes by norepinephrine or endothelin, which may be upregulated by proinflammatory stimuli or ischemia. LOX-1 on the cell surface of cardiac myocytes might possibly be another source of sLOX-1.

Although LOX-1 expression was prominent in atherosclerotic lesions and remarkably inducible by proinflammatory stimuli, serum sLOX-1 did not reflect just general inflammation or atherosclerotic lesion sizes but rather instability of atherosclerotic plaques. In fact, sLOX-1 was elevated in the acute phases of ACS, but not in general acute inflammatory diseases in which serum hs-CRP levels were high (Figure 1). In addition, serum sLOX-1 levels were not significantly correlated with those of the inflammatory marker hs-CRP or numbers of affected coronary arteries (data not shown). Although a recent report has shown that CRP can induce LOX-1 expression, LOX-1 can also be induced by a variety of biological stimuli, and regulation of LOX-1 cleavage may not be so correlated with CRP. Circulating Ox-LDL levels, which might be mildly oxidized, have been reported to be elevated in ACS, although its sensitivity or specificity for the diagnosis of ACS was not demonstrated. CRP. The antibodies used in our ELISA can be bound to sLOX-1 in the presence of Ox-LDL; in fact, the addition of Ox-LDL to sLOX-1 samples did not affect the results of our sLOX-1 ELISA (see the Table in the online-only Data Supplement). Therefore, Ox-LDL in serum does not appear to interfere with the results of our sLOX-1 ELISA.

In addition, sLOX-1 did not show any correlation with TnT (Figure 3B) or CKP, suggesting that sLOX-1 is not a marker for cardiac necrosis or injury. Furthermore, peak time of sLOX-1 in serum was earlier than that of TnT (Figure 3A). This is quite reasonable because plaque instability or rupture precedes cardiac necrosis or ischemic injury and suggests that sLOX-1 appears to be a suitable serum marker for early diagnosis of ACS, especially NQ-ACS without severe cardiac necrosis or damage. In fact, sLOX-1 showed higher sensitivity for early detection of NQ-ACS than TnT or hs-CRP did (Table 3). Moreover, even in ACS patients without significant elevation of TnT levels (<0.03 ng/mL) at the time of visit, 86% of these TnT-negative patients showed sLOX-1 levels >1.0 ng/mL (Table 3), indicating the usefulness of sLOX-1 measurement, in addition to TnT, at the very early stage.

We currently do not know exactly when serum sLOX-1 levels begin to increase before the onset of ACS; however, sLOX-1 levels at the time of visit showed almost the peak values for each patient (Figure 3A), suggesting that serum sLOX-1 levels may begin to rise before the onset of ACS. Further large-scale prospective studies will tell us more about the value of serum sLOX-1 for predicting ACS onset.

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References


Serum Soluble Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 Levels Are Elevated in Acute Coronary Syndrome: A Novel Marker for Early Diagnosis

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On-line Figure

Dilution of human serum samples in soluble LOX-1 ELISA
A patient's serum sample, of which the soluble LOX-1 concentration was more than 10 ng/mL, was subjected to soluble LOX-1 ELISA after serial dilution (1/2, 1/4, and 1/8). Diluted serum samples are indicated as open (white) circles, and the soluble LOX-1 concentration standards are shown as closed (black) circles. Results: Open circles are overlapped with the standard curve (closed circles), indicating the accuracy of this soluble LOX-1 ELISA.