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Original article

Soluble lectin-like oxidized LDL receptor-1 (sLOX-1) as a sensitive and specific biomarker for acute coronary syndrome—Comparison with other biomarkers

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Summary
Background: Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) appears to be involved in atherosclerotic plaque vulnerability and rupture. Circulating soluble LOX-1 (sLOX-1) levels are dramatically elevated in patients with acute coronary syndrome (ACS), and its diagnostic sensitivity and specificity is superior to high-sensitivity C-reactive protein (hs-CRP). In this study, we have compared the diagnostic value of sLOX-1 for ACS with those of troponin T (TnT) and heart-type fatty acid binding protein (H-FABP).
Methods: One hundred and seven patients who underwent coronary angiography (CAG), including 18 ACS and 89 non-ACS patients were enrolled. Peripheral blood samples were obtained during the emergent or elective CAG. The non-ACS group consisted of 30 patients with normal CAG, 30 stable angina pectoris patients controlled by medical treatment, and 29 patients with stable angina who required elective coronary revascularization (percutaneous coronary intervention or coronary artery bypass graft).
Results: Age, gender, lipid profiles, or prevalence of diabetes, smoking, or hypertension were not significantly different between ACS and non-ACS. These factors did not significantly affect blood sLOX-1 levels. Circulating sLOX-1, TnT, and H-FABP levels were significantly higher in ACS than non-ACS. Area under the curve (AUC) values of the receiver-operating characteristic curves

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were 0.948, 0.704, and 0.691 for sLOX-1, TnT, and H-FABP, respectively. In a TnT-negative (<0.03 ng/mL) subgroup, the AUC values for sLOX-1 and H-FABP were 0.848 and 0.476, respectively.
Conclusion: Circulating sLOX-1 is a more sensitive and specific biomarker for ACS than TnT and H-FABP, and provides additional diagnostic values when measured in combination with TnT.

Introduction

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 the prognosis by appropriate treatment without delay. ACS appears to be provoked by rupture, or erosion, of lipid-rich atheromatous plaques followed by thrombus formation [1,2]. Several diagnostic tests, such as electrocardiogram (ECG), echocardiography [3], radioisotope scintigraphy [4], and measurement of circulating levels of MB isoform of creatine kinase (CPK) [5], troponin T (TnT) [6,7], and heart-type fatty acid binding protein (H-FABP) [8—10] have been utilized to detect ischemic myocardial damage in clinical practice. However, TnT or H-FABP may not always be elevated at the earliest stages of ACS, especially in cases of non-ST-elevation ACS (NSTE-ACS), before myocardial necrosis or ischemic damage becomes apparent. Certain biomarkers for plaque instability or rupture would make it possible to diagnose ACS from the earliest stage, and, furthermore, may predict the ACS onset.

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a receptor for atherogenic oxidized low-density lipoprotein (Ox-LDL), whose expression is not constitutive but dynamically inducible by proinflammatory stimuli, angiotensin II, and Ox-LDL, which affects atherosclerotic progression and plaque vulnerability [11—17]. In human atherosclerotic lesions, LOX-1 is prominently expressed by intimal smooth muscle cells and lipid-laden macrophages in advanced plaques [18]. Furthermore, LOX-1 plays an important role in Ox-LDL-induced apoptosis of vascular endothelial and smooth muscle cells [19—21] and production of matrix metalloproteinases [22], which may thus directly be linked to the plaque vulnerability and rupture. Furthermore, LOX-1 expression was associated with the instability of atherosclerotic plaques in a hypercholesterolemic animal model [23—25].

LOX-1 expressed on the cell-surface can be proteolytically cleaved at its membrane-proximal extracellular domain and released as soluble LOX-1 (sLOX-1) [26]. Cleavage of LOX-1 from the cell-surface appears to be mediated by certain metalloproteinases, including ADAM10, and to be regulated dynamically by proinflammatory stimuli [27]. In previous reports, we have shown that circulating sLOX-1 levels are significantly elevated in ACS from its early stage [28,29]. Furthermore, receiver-operating characteristic (ROC) curves showed that diagnostic sensitivity and specificity of sLOX-1 for ACS were much higher than those of high-sensitivity C-reactive protein (hs-CRP) [28]. In addition, the diagnostic sensitivity and specificity were almost equal even in NSTE-ACS alone [28]. The present study, therefore, has further compared diagnostic sensitivity and specificity with those of TnT and H-FABP, both of which are currently used as diagnostic blood biomarkers for ACS in clinical practice.

Materials and methods

Patient sample

One hundred and seven patients (18 ACS and 89 non-ACS) undergoing coronary angiography (CAG), from whom informed consent and blood samples were obtained and their TnT and H-FABP date were available, at the Cardiovascular Center in Osaka Red Cross Hospital were enrolled. Peripheral blood samples were obtained immediately after emergent (ACS) or scheduled (non-ACS) CAG. ACS was defined as acute onset of prolonged chest pain or discomfort, which was 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, as previously described [28]. The non-ACS group ($n = 89$) consisted of 30 patients without any significant luminal narrowing in CAG, 30 stable angina pectoris patients controlled by medical treatment, and 29 patients with stable angina who required elective coronary revascularization (percutaneous coronary intervention or coronary artery bypass graft) [28]. Patients with vasospastic angina, symptomatic peripheral vascular diseases, or stroke were excluded from this study. Informed consent was obtained from the involved patients. This study was carried out in accordance with the principles of the declaration of Helsinki and had been approved by the local ethics committee.

Measurement of soluble LOX-1 and other biomarkers

Peripheral blood samples were obtained immediately after emergent or scheduled CAG. Serum was obtained by centrifugation, and samples were stored at −80 °C. Concentrations of sLOX-1 were determined by a sandwich chemiluminescent enzyme immunoassay (CLEIA) using two different human LOX-1-specific monoclonal antibodies as recently described [30]. Standard curves were obtained by the use of a recombinant protein corresponding to the extracellular domain of human LOX-1. This CLEIA showed sufficient correlation with our previously described sand-

Table 1 Characteristics of patients.

	Total	ACS	Non-ACS	*p*-Value
Patient numbers	107	18	89	
Age (years)	64.2 ± 11.4	62.6 ± 14.3	65.3 ± 10.3	0.359
Male (%)	66.7	66.9	65.7	0.786
Diabetes (%)	30.5	35.3	29.6	0.774
Smoking (%)	35.2	41.2	34.1	0.589
Hypertension (%)	43.0	23.1	46.9	0.144
Total cholesterol (mg/dL)	191.5 ± 34.8	174.6 ± 34.9	194.0 ± 3 4.3	0.0845
Triglycerides (mg/dL)	129.7 ± 61.3	104.4 ± 30.7	133.5 ± 64.0	0.125
HDL cholesterol (mg/dL)	47.2 ± 14.0	43.6 ± 11.0	47.8 ± 14.3	0.335
LDL cholesterol (mg/dL)	117.8 ± 32.7	113.5 ± 34.4	118.5 ± 32.7	0.623

Values are mean ± S.D. ACS, acute coronary syndrome; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

wich enzyme-linked immunosorbent assay (ELISA) by the use of two polyclonal antibodies [28,29], and the lower limit of the detection for sLOX-1 was 0.008 ng/mL [30]. TnT and H-FABP concentrations were measured by commercially available electro-chemiluminescent immunoassay (ECLIA, Roche Diagnostic, Basel, Switzerland) and ELISA (Marco Pharmaceutical, Tokyo, Japan), respectively. All the assays were carried out by personnel who had no knowledge of the clinical diagnosis of the patients.

Statistical analysis

The distribution of sLOX-1, TnT, and H-FABP was skewed; therefore, Wilcoxon/Kruskal—Wallis test was utilized. ANOVA was applied for other clinical parameters. Association of sLOX-1 with other values, including TnT and H-FABP, was evaluated by Spearman's rank-correlation coefficient. ROC analysis was carried out on the levels of sLOX-1, TnT, and H-FABP for ACS with non-ACS as a negative control group. This analysis plots the true positive fraction (sensitivity) against the false positive fraction (1-specificity) by changing the cut-off value for the test. Areas under the ROC curves indicate the relative accuracy of diagnostic tests. *p*-Values less than 0.05 were considered statistically significant.

Results

Clinical characteristics of study subjects

Table 1 summarizes the clinical characteristics of the subjects enrolled in this study. Age, gender, conventional cardiovascular risk factors, such as diabetes, smoking hypertension, and lipid profiles, were compared between ACS and non-ACS groups. Age (62.6 ± 14.3 years versus 65.3 ± 10.3 years, $p = 0.359$), gender (male %: 66.9% versus 65.7%, $p = 0.786$), or prevalence of diabetes (35.3% versus 29.6%, $p = 0.774$), smoking (41.2% versus 34.1%, $p = 0.589$) or hypertension (23.1% versus 46.9%, $p = 0.144$) were not significantly different between the ACS and non-ACS groups. In addition, neither serum total cholesterol (174.6 ± 34.9 mg/dL versus 194.0 ± 34.3 mg/dL, $p = 0.0845$), triglyceride (104.4 ± 30.7 mg/dL versus 133.5 ± 64.0 mg/dL, $p = 0.125$), high-density lipoprotein (HDL) cholesterol (43.6 ± 11.0 mg/dL versus 47.8 ± 14.3 mg/dL, $p = 0.335$) nor low-density lipoprotein (LDL) cholesterol (113.5 ± 34.4 mg/dL versus 118.5 ± 32.7 mg/dL, $p = 0.623$) levels were significantly different between ACS and non-ACS groups.

Conventional cardiovascular risk factors and lipid profiles versus soluble LOX-1, TnT and H-FABP

The correlation of blood biomarkers, such as sLOX-1, TnT, and H-FABP, with conventional cardiovascular risk factors and serum lipid profiles was explored. As shown in Table 2, circulating sLOX-1 levels were not significantly correlated with age (Spearman's $\rho = 0.0304$, $p = 0.758$), total cholesterol (Spearman's $\rho = -0.184$, $p = 0.937$), triglycerides (Spearman's $\rho = -0.133$, $p = 0.211$), HDL cholesterol (Spearman's $\rho = -0.141$, $p = 0.185$) or LDL cholesterol (Spearman's $\rho = -0.0223$, $p = 0.827$). In addition, sLOX-1 levels were not significantly different between male or female (median: 0.121 versus 0.101 ng/mL, $p = 0.370$) or between with and without conventional risk factors, such as diabetes (median: 0.114 versus 0.104 ng/mL, $p = 0.895$), smoking (median: 0.101 versus 0.122 ng/mL, $p = 0.641$), or hypertension (median: 0.104 versus 0.098 ng/mL, $p = 0.522$) (Table 3). On the other hand, TnT levels were not significantly correlated with age (Spearman's $\rho = 0.0133$, $p = 0.893$) although H-FABP levels were significantly correlated with age (Spearman's $\rho = 0.201$, $p = 0.0398$), as previously described [31]. In addition, neither TnT nor H-FABP levels were significantly correlated with total cholesterol (TnT: Spearman's $\rho = -0.134$, $p = 0.223$; H-FABP: Spearman's $\rho = 0.150$, $p = 0.174$), triglycerides (TnT: Spear-

Table 2 Correlation of soluble lectin-like oxidized low-density lipoprotein receptor-1 with age and lipid profiles.

	Spearman ρ	*p*-value
Age	0.0304	0.758
Total cholesterol	−0.184	0.0937
Triglycerides	−0.133	0.211
HDL cholesterol	−0.141	0.185
LDL cholesterol	−0.0223	0.827

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3 Comparison of soluble lectin-like oxidized low-density lipoprotein receptor-1 levels between male and female or with and without conventional risk factors.

	Median (25th—75th percentiles)		*p*-Value
	Yes	No	
Male	0.121 (0.073—0.216)	0.101 (0.050—0.262)	0.370
Diabetes	0.114 (0.054—0.280)	0.104 (0.072—0.204)	0.895
Smoking	0.101 (0.073—0.187)	0.122 (0.067—0.266)	0.641
Hypertension	0.104 (0.051—0.165)	0.098 (0.068—0.314)	0.522

man's $\rho = -0.148$, $p = 0.163$; H-FABP: Spearman's $\rho = 0.0195$, $p = 0.854$), HDL cholesterol (TnT: Spearman's $\rho = -0.0907$, $p = 0.395$; H-FABP: Spearman's $\rho = 0.0579$, $p = 0.588$) or LDL cholesterol (TnT: Spearman's $\rho = -0.0347$, $p = 0.733$; H-FABP: Spearman's $\rho = 0.0823$, $p = 0.418$). Furthermore, neither TnT nor H-FABP levels were significantly different between males and females (TnT median: 0.01 ng/mL versus 0.01 ng/mL, $p = 0.982$; H-FABP median: 2.95 ng/mL versus 4.1 ng/mL, $p = 0.116$) or between with and without conventional risk factors, such as diabetes (TnT median: 0.01 ng/mL versus 0.01 ng/mL, $p = 0.1584$; H-FABP median: 3.5 ng/mL versus 3.3 ng/mL, $p = 0.821$), smoking (TnT median: 0.01 ng/mL versus 0.01 ng/mL, $p = 0.873$), and hypertension (TnT median: 0.01 ng/mL versus 0.01 ng/mL, $p = 0.0975$; H-FABP median: 3.6 ng/mL versus 2.9 ng/mL, $p = 0.441$), except that H-FABP levels were significantly lower in smokers than non-smokers (median: 2.9 ng/mL versus 3.8 ng/mL, $p = 0.0195$) in this study population.

Soluble LOX-1, TnT, and H-FABP levels in ACS

In ACS cases, time intervals from the symptom onset to the blood sampling were 4.8 ± 4.3 h in this study. As shown in Fig. 1, peripheral blood sLOX-1, TnT, and H-FABP levels were remarkably higher in ACS than non-ACS (sLOX-1 median, 25th and 75th percentiles: 1.13, 0.168, and 3.46 versus 0.096, 0.0645, and 0.162 ng/mL, $p < 0.0001$; TnT median, 25th and 75th percentiles: 0.04, 0.01, and 0.36 versus 0.01, 0.01, and 0.01 ng/mL, $p = 0.0003$; H-FABP median, 25th and 75th percentiles: 7.8, 2.9, and 100.5 versus 3.3, 2.5, and 4.9 ng/mL, $p = 0.012$). These differences were statistically significant as previously reported [6—10,28—30]. To compare diagnostic sensitivity and specificity for ACS, ROC curves were compared among sLOX-1, TnT, and H-FABP (Fig. 2). Area under the ROC curve (AUC) values were 0.948, 0.704, and 0.691 for sLOX-1, TnT, and H-FABP, respectively, indicating that sLOX-1 showed the highest sensitivity and specificity for ACS among these three biomarkers. Furthermore, ROC curves for the diagnosis of STE-ACS alone were compared, among sLOX-1, TnT, and H-FABP, by exclusion of NSTE-ACS. Among 18 ACS patients, 13 subjects were STE-ACS and 5 cases were NSTE-ACS. ROC curves for STE-ACS (Fig. 3) were not so much different from those for whole ACS (Fig. 2). The AUC values were 0.920, 0.731, and 0.633 for sLOX-1, TnT, and H-FABP, respectively.

In the ACS group, H-FABP values were significantly correlated with TnT values (Spearman's $\rho = 0.658$, $p = 0.003$). In contrast, sLOX-1 values were not significantly correlated with TnT (Spearman's $\rho = 0.176$, $p = 0.499$) or H-FABP (Spearman's $\rho = 0.343$, $p = 0.178$) values, indicating that sLOX-1 is independent of TnT and H-FABP. To further evaluate the diagnostic values of sLOX-1, ROC curves of sLOX-1 and H-FABP were compared in a subpopulation whose TnT levels were below 0.03 ng/mL (an optimal cut-off value for ACS diagnosis). This cut-off value showed 50% sensitivity and 83% specificity for ACS diagnosis in this study population, as shown in Fig. 2B. In this TnT-negative subgroup (83 subjects in total), 9 cases were ACS and 74 subjects were non-ACS. As shown in Fig. 4, diagnostic sensitivity and specificity were much superior in sLOX-1; the AUC values for sLOX-1 and H-FABP, in this TnT-negative subgroup, were 0.848 and 0.476, respectively. Thus, sLOX-1 shows significant diagnostic values for ACS in addition to TnT; however, H-FABP may not appear to show any additional diagnostic values when measured in combination with TnT at the same time.

Discussion

Rupture or erosion of atheromatous plaques followed by thrombus formation is considered as a crucial step in the pathogenesis of ACS. Atherosclerotic plaques with abundant lipid-laden macrophages and activated smooth muscle cells with proinflammatory stimuli in the intima appear to be prone to rupture [1,2]. In such vulnerable plaques, LOX-1 is prominently expressed by smooth muscle cells and macrophages [18] and contributes to apoptosis of endothelial and smooth muscle cells [19—21] and production of matrix metalloproteinases [22]. Under these conditions, enhanced protease activities may cleave sLOX-1 from the surface of these vascular cells, in which LOX-1 is abundantly expressed. Our recent studies have indicated that ADAM family proteases may be involved in LOX-1 cleavage and that proinflammatory stimuli can enhance this cleavage process [27]. Therefore, the rationale for this test to detect ACS at the earliest stage is that plaque instability or rupture would precede the ischemic myocardial damage that releases TnT and H-FABP. Additionally, elevated levels of sLOX-1 may also reflect platelet activation and thrombus formation followed by plaque erosion or rupture [32,33]. As a biomarker for platelet activation and thrombus formation, utility of D-dimer has also been suggested [34].

In the present study, we compared diagnostic values of sLOX-1 with those of current biomarkers for ACS, such as TnT and H-FABP in the same blood samples, and revealed that sLOX-1 shows the highest diagnostic sensitivity and specificity. In addition, sLOX-1 was able to detect ACS in subjects whose TnT levels were not significantly elevated, indicating that diagnostic accuracy would be significantly improved

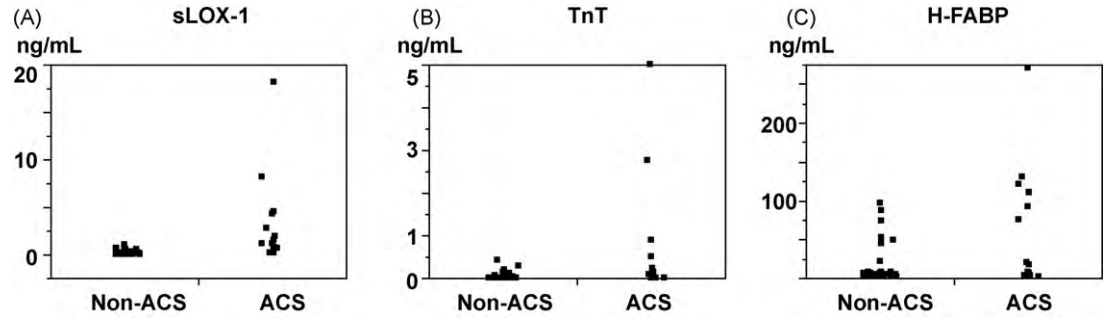

Figure 1 Comparison of soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1), troponin T (TnT), and heart-type fatty acid-binding protein (H-FABP) levels between acute coronary syndrome (ACS) and non-ACS. Peripheral blood sLOX-1, TnT, and H-FABP levels were compared between 18 ACS and 89 non-ACS subjects at the acute stage. Levels of sLOX-1 ($p < 0.0001$), TnT ($p = 0.0003$), and H-FABP ($p = 0.012$) were significantly higher in ACS than non-ACS.

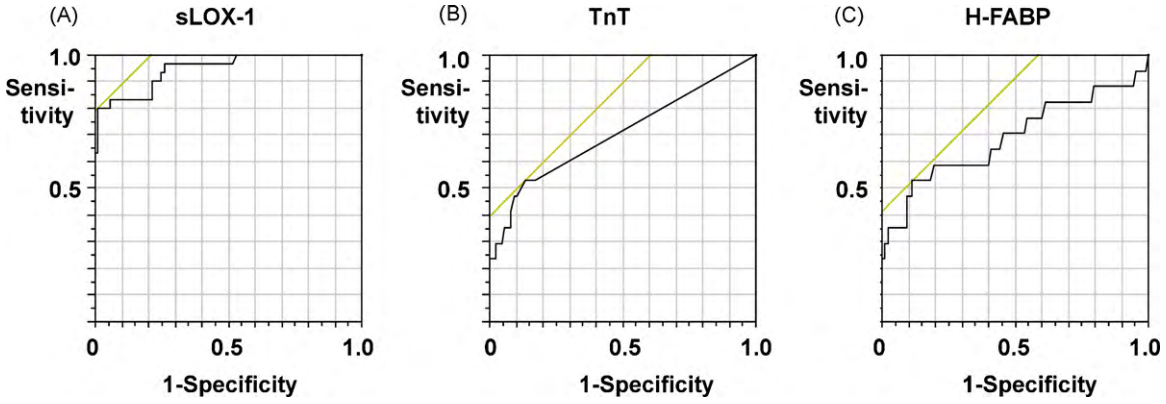

Figure 2 Receiver-operating-characteristics (ROC) curves of soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) (A), troponin T (TnT) (B), and heart-type fatty acid-binding protein (H-FABP) (C) for the diagnosis of acute coronary syndrome (ACS). The true positive fraction (sensitivity as Y axis) was plotted against the false positive fraction (1-specificity as X axis) by changing the cut-off values of sLOX-1 (A), TnT (B), and H-FABP (C) for the diagnosis of ACS. Area under the ROC curve values were 0.948, 0.704, and 0.691 for sLOX-1, TnT, and H-FABP, respectively.

when TnT and sLOX-1 are measured in combination. However, the limitation of this study appears to be the lack of time-window analyses for the each biomarker during the acute stage of ACS, because of the sample size and the single blood sampling. It, therefore, would be necessary, in the future, to evaluate the diagnostic values in larger samples including those with acute chest symptoms in emergency rooms, comparing the results among different time-windows

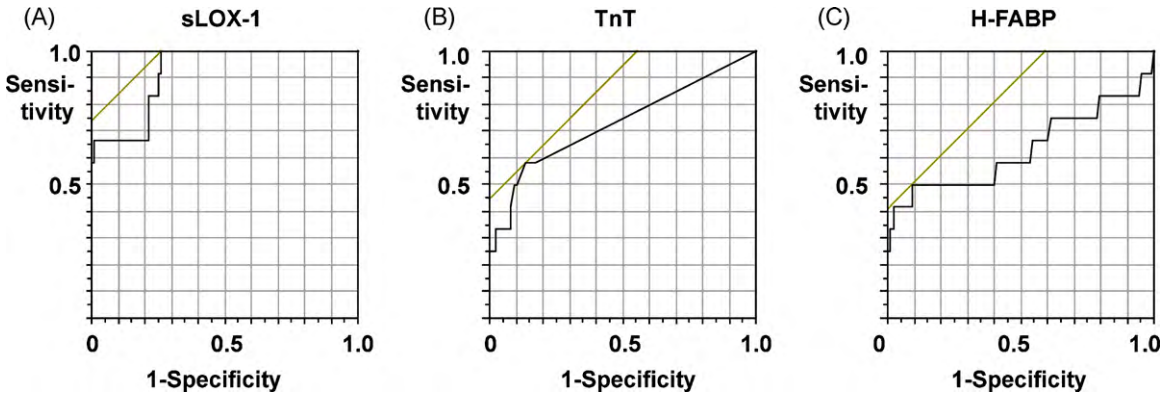

Figure 3 Receiver-operating-characteristics (ROC) curves of soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) (A), troponin T (TnT) (B) and heart-type fatty acid-binding protein (H-FABP) (C) for the diagnosis of ST-elevation acute coronary syndrome (STE-ACS). The true positive fraction (sensitivity as Y axis) was plotted against the false positive fraction (1-specificity as X axis) by changing the cut-off values of sLOX-1 (A), TnT (B), and H-FABP (C) for the diagnosis of STE-ACS by excluding non-STE-ACS. Area under the ROC curve values were 0.920, 0.731, and 0.633 for sLOX-1, TnT, and H-FABP, respectively.

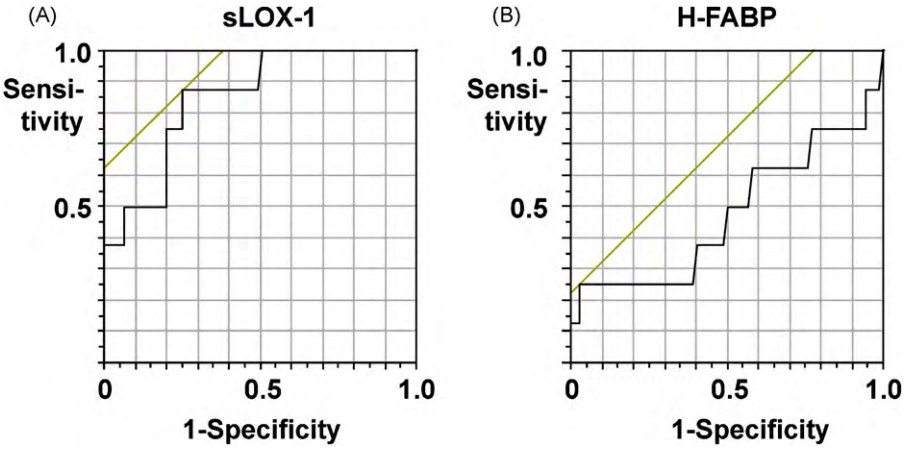

Figure 4 Comparison of receiver-operating-characteristics (ROC) curves for the diagnosis of acute coronary syndrome (ACS) between soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) and heart-type fatty acid-binding protein (H-FABP) in the troponin T (TnT)-negative subpopulation. In the TnT-negative (<0.03 ng/mL) subpopulation, ROC curves of sLOX-1 (A) and H-FABP (B) for ACS are indicated. Area under the ROC curve values were 0.848 and 0.476 for sLOX-1 and H-FABP, respectively.

from the symptom onset. In addition, diagnostic sensitivity and specificity of sLOX-1 might be inferior to TnT at more than 12 h after the onset of ACS, as we have previously indicated the time-dependent changes in sLOX-1 and TnT levels after the onset of ACS [28]. Recently, high-sensitivity troponins T and I have been shown to detect acute myocardial infarction (AMI) at the early stages [35,36]. In these large-scale international multicenter studies, the ROC curve of the conventional TnT to diagnose AMI appeared similar to ours, thus indicating the appropriateness of our patient samples although the size was small. Therefore, it should be tested, in the future, whether sLOX-1 has incremental values to diagnose ACS or AMI at the early stages in addition to these sensitive assays for troponins.

At present, furthermore, we do not know exactly when circulating sLOX-1 levels begin to be elevated before the onset of ACS. In our previous study, however, sLOX-1 levels at the initial time of visit showed almost the peak values for each patient [28]. Therefore, peripheral blood sLOX-1 levels may begin to rise before the onset of ACS in advance. Further large-scale prospective studies, in the future, would also elucidate the predictive values of circulating sLOX-1 for the onset of ACS or AMI.

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