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ORIGINAL ARTICLE
Ischemic Heart Disease

Soluble Lectin-Like Oxidized LDL Receptor-1 and High-Sensitivity Troponin T as Diagnostic Biomarkers for Acute Coronary Syndrome

– Improved Values With Combination Usage in Emergency Rooms –

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Background: Although highly sensitive assays for troponin T (hs-TnT) have been developed, the sensitivity and specificity of hs-TnT for diagnosing acute coronary syndrome (ACS) remains imperfect. We evaluated the diagnostic value of a new biomarker of plaque vulnerability (soluble lectin-like oxidized low-density lipoprotein receptor-1, sLOX-1) as compared with hs-TnT in the emergency room (ER).

Methods and Results: Plasma sLOX-1 and serum hs-TnT levels were measured in 200 consecutive patients presenting with chest symptoms and ECG abnormalities in the ER (116 ST elevation ACS [STEACS], 44 non-ST elevation ACS [NSTEACS], 40 non-ACS). The non-ACS group consisted of patients with cardiovascular diseases such as coronary spastic angina pectoris, pulmonary thromboembolism, perimyocarditis and takotsubo cardiomyopathy. Levels of sLOX-1 and hs-TnT were significantly higher in STEACS and NSTEACS than in non-ACS patients. The receiver-operating characteristic (ROC) curves of sLOX-1 and hs-TnT for detecting ACS, using the non-ACS patients as negative references, showed that the area under the curve (AUC) values of sLOX-1 and hs-TnT were 0.769 and 0.739, respectively. In the lower hs-TnT (<0.0205 ng/ml) subgroup, the AUC value of the ROC curve of sLOX-1 for detecting ACS was 0.869.

Conclusions: The diagnostic value for ACS was comparable between sLOX-1 and hs-TnT, and the accuracy of ACS diagnosis appeared to improve when sLOX-1 and hs-TnT were measured in combination. (*Circ J* 2011; **75:** 2862–2871)

Key Words: High-sensitivity troponin T; Myocardial damage; Plaque instability; Plaque rupture; Soluble lectin-like oxidized low-density lipoprotein receptor-1

Although acute myocardial infarction (AMI) can be diagnosed using biomarkers of myocardial necrosis or damage, such as creatine kinase-MB (CK-MB)[1] and standard assay cardiac troponins,[2–6] none of these is an early marker of myocardial necrosis because the elevation of these markers begins 3–4 h after the onset of AMI. Highly sensitive assays for cardiac troponins have been developed[7,8] and have become the gold standard for diagnosing AMI in recent years, because of improved diagnostic sensitivity for early-stage acute coronary syndrome (ACS).[9,10] However, the increase in sensitivity has been associated with a decrease in specificity,[11] and therefore the diagnostic sensitivity for ACS in the early stage has remained imperfect because of the optimal cut-off value.[10]

The specificity of highly sensitive cardiac troponins for diagnosing ACS in the real clinical setting is insufficient because these troponins are also elevated in patients with diseases other than ACS that are associated with minimal myocardial damage, such as coronary spastic angina pectoris (CSA), pulmonary thromboembolism, perimyocarditis (pericarditis and/or myocarditis) and takotsubo cardiomyopathy. It is difficult to differentiate ACS from these diseases because all of

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Table 1. Characteristics of All Enrolled Patients

	ACS		Non-ACS (n=40)	P value
	STEACS (n=116)	NSTEACS (n=44)		
Male, n (%)	105 (91)*,†	30 (68)*	25 (63)†	<0.001
Age (years), mean±SD	64±12	66±10	66±13	0.700
Risk factors, n (%)				
Diabetes	40 (34)‡	11 (25)§	3 (8)‡,§	0.004
Hypertension	71 (61)#	34 (77)‡‡	15 (38)#,‡‡	<0.001
Dyslipidemia	74 (64)	29 (66)	20 (50)	0.240
Smoking	75 (65)¶	21 (48)	14 (35)¶	0.003
Prehospital drug use				
ACEI/ARB	21 (18)	6 (14)	4 (10)	0.440
Statin	9 (8)	8 (18)	4 (10)	0.188
Creatinine (mg/dl), mean±SD	0.90±0.28	0.91±0.48	0.83±0.27	0.389
Peak CK-MB (IU/L), median (25–75th percentiles)	124 (66–228)	18 (11–40)	7 (5–10)	<0.001
Interval from the onset of symptoms to ER arrival (min), median (25–75th percentiles)	146 (82–416)**,††	296 (125–900)**	310 (105–1,145)††	0.010
Culprit vessel				
Right coronary artery, n (%)	44 (38)	9 (20)		0.036
Left anterior descending artery, n (%)	62 (53)	26 (59)		0.522
Left circumflex artery, n (%)	10 (9)	9 (20)		0.039
No. of diseased vessels, mean±SD	1.76±0.81	1.71±0.77		0.539

By the χ^2 test: *P<0.001 between STEACS and NSTEACS; †P<0.001 between STEACS and non-ACS; ‡P=0.001 between STEACS and non-ACS; §P=0.032 between STEACS and non-ACS; #P<0.001 between STEACS and non-ACS; ‡‡P<0.001 between STEACS and non-ACS, ¶P=0.001 between STEACS and non-ACS.
By the Mann Whitney U test: **P=0.013 between STEACS and NSTEACS. ††P=0.019 between STEACS and non-ACS.
ACS, acute coronary syndrome; STEACS, ST elevation acute coronary syndrome; NSTEACS, non-STE acute coronary syndrome; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; CK, creatine kinase; ER, emergency room.

them exhibit similar chest symptoms and ECG abnormalities, as well as subtle increases in biomarkers for cardiac damage. In fact, the usefulness of cardiac biomarkers for these differential diagnoses has not been fully reported yet.

Lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) is a receptor for atherogenic oxidized LDL, which is abundantly expressed in advanced human atherosclerotic plaques.[12] It is cleaved at the membrane-proximal extracellular domain by proteases, and released as a soluble form.[13,14] Therefore, soluble LOX-1 (sLOX-1) is regarded as a marker of plaque rupture or instability. Our previous studies revealed the diagnostic value of sLOX-1 for early-stage ACS in which conventional troponin T (TnT) was unable to provide a diagnosis.[13,15–19] However, the diagnostic value of sLOX-1 has not been compared with the highly sensitive form of TnT (hs-TnT) in the emergency room (ER), where ACS needs to be distinguished from non-ACS with similar acute chest symptoms and ECG abnormalities.

The aim of the present study was to evaluate the diagnostic value of sLOX-1 and hs-TnT for patients in the ER with chest symptoms suggestive of ACS and ECG abnormalities.

Methods

Study Population

Between April 2008 and December 2009 we assigned 200 consecutive patients (160 males, 40 females; mean age, 65±12 years) presenting at the ER of Nippon Medical School Chiba Hokusoh Hospital with chest symptoms and ECG abnormalities within 48 h of the onset of chest symptoms, which were defined as chest pain, chest oppression and chest discomfort suggestive of ACS. ECG abnormalities were defined as ST segment elevation or depression (≥0.05 mV), and/or T wave inversion (≥0.05 mV), in 2 or more contiguous leads on the ECG. ACS was defined as prolonged chest pain (≥20 min) with a coronary artery lesion on coronary angiography. STEACS and NSTEACS were respectively defined as ACS with and without ST segment elevation (≥0.05 mV) in 2 or more contiguous leads on ECG. ACS, STEACS and NSTEACS within 3 h of the onset of chest symptoms were all defined as "early". Patients undergoing renal replacement therapy for either acute or chronic kidney disease, and those on a percutaneous cardiopulmonary support system for cardiogenic shock or fatal arrhythmia were excluded from the study.

Measurement of sLOX-1 and hs-TnT

Sampling Methods and Biomarker Measurements Peripheral blood samples were obtained in the ER, and the plasma samples were separated by centrifugation and stored frozen at –80°C. Plasma sLOX-1 levels were measured using a sandwich chemiluminescent enzyme immunoassay (CLEIA) with 2 newly-developed anti-human LOX-1 monoclonal antibodies,[17] which a modification of a previously described enzyme-linked immunosorbent assay (ELISA) using the former polyclonal antibodies.[16] Intra- and interassay coefficients of variation (CV) were reported to be 4.4–6.5% and 4.0–6.1%, respectively, and the lower limit of detection for sLOX-1 was 8.0 pg/ml.[17] Serum hs-TnT levels were measured using an electrochemoluminescence immunoassay (ECLusys Troponin hs; Roche Diagnostics Co, Tokyo, Japan). Intra- and interassay CVs were reported to be 0.5–5.7% and 1.4–3.0% ng/ml, respectively, and the lower limit of the detection for hs-TnT was 0.003 ng/ml.[20]

Statistical Analysis

Continuous variables are presented as means±standard deviation and were compared between 2 groups by Student's t-test

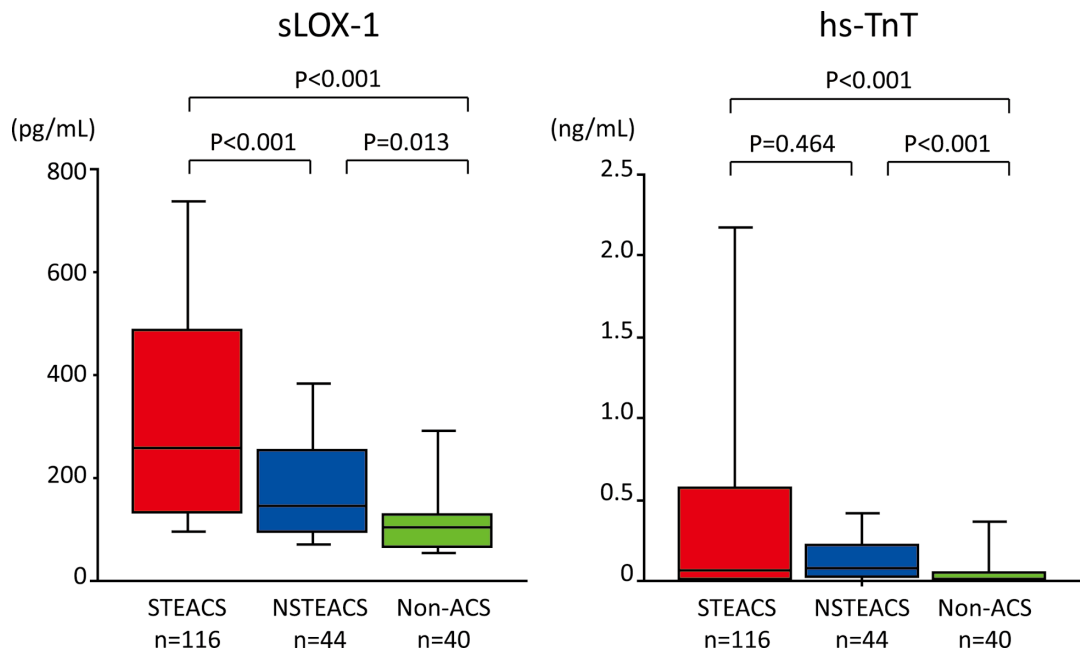

Figure 1. Plasma levels of soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) and serum high-sensitivity troponin T (hs-TnT) were compared in consecutive patients with chest symptoms and ECG abnormalities in the emergency room among groups of patients with ST elevation acute coronary syndrome (STEACS, n=116), non-STE ACS (NSTEACS, n=44) and non-ACS, n=44). Center horizontal lines indicate median values. Lower and upper edges of boxes indicate 25th and 75th percentiles, and lower and upper bars indicate 10th and 90th percentiles, respectively.

and among 3 groups by 1-way ANOVA. Dichotomous variables were compared using χ^2 statistics. Because sLOX-1, hs-TnT, CK-MB and time intervals were not normally distributed, these values are presented as medians and 25th and 75th percentiles, and were compared by Mann Whitney U test and Kruskal-Wallis test for 2 and 3 groups, respectively. The correlation of the plasma sLOX-1 or serum hs-TnT level in the ER with the time interval from the onset of chest symptoms to ER arrival was analyzed by Spearman's rank correlation test. To compare the sensitivities and specificities of sLOX-1 and hs-TnT for diagnosing ACS, receiver-operating characteristics (ROC) curve analyses were carried out using the non-ACS subjects as negative controls. ACS patients whose sLOX-1 and hs-TnT values were less than the optimal cut-off values determined from the ROC curves to diagnose ACS were defined as the sLOX-1-negative ACS subgroup and hs-TnT-negative ACS subgroup, respectively. ACS patients whose sLOX-1 and hs-TnT values were more than those optimal cut-off values, were defined as sLOX-1-positive ACS subgroup and hs-TnT positive ACS subgroup, respectively. Data were statistically analyzed using the SPSS software package, version 16.0. A P-value <0.05 was considered statistically significant. The Ethics Committee of the hospital approved the study protocols, and written informed consent to participate in the investigations was given by all of the patients.

Results

Characteristics of the Enrolled Patients

The 200 consecutive patients enrolled in this study consisted of 160 patients with ACS (116 STEACS, 44 NSTEACS) and 40 patients with non-ACS (23 CSA, 6 arrhythmia, 6 pulmonary thromboembolism, 3 perimyocarditis, 2 takotsubo cardiomyopathy). Their characteristics are shown in Table 1. Age, prevalence of dyslipidemia, and serum creatinine levels were comparable among the STEACS, NSTEACS and non-ACS groups. Prevalence of prehospital use of angiotensin-converting inhibitors (ACEI)/angiotensin-receptor blockers (ARB), and statins was also comparable among the 3 groups. Risk factors, including diabetes, hypertension and smoking habit, were comparable between the STEACS and NSTEACS groups, but were more prevalent in patients with ACS than in the non-ACS group. Males were more prevalent in the STEACS group than in the NSTEACS and non-ACS groups. Peak serum CK-MB levels were significantly higher in the STEACS and NSTEACS groups than in the non-ACS group (P<0.001), and significantly higher in the STEACS group than in the NSTEACS group (P<0.001). The time interval from the onset of chest symptoms to ER arrival was significantly shorter in the STEACS group than in the NSTEACS (P=0.013) and non-ACS (P=0.019) groups. The time intervals was comparable between the NSTEACS and non-ACS groups (P=0.914). Concerning the culprit vessel in ACS, the incidence of left anterior descending artery lesions was comparable between the STEACS and NSTEACS groups; however, right coronary artery lesions were more prevalent in the STEACS group, and left circumflex lesions were more prevalent in the NSTEACS group. The number of diseased vessels was comparable between the 2 ACS groups.

Comparison of sLOX-1 and hs-TnT Levels

As shown in Figure 1, the median and 25th and 75th percentile values of plasma sLOX-1 were 259.0, 134.5 and 488.9 pg/ml for STEACS, 143.9, 96.6 and 255.1 pg/ml for NSTEACS, and

Table 2. Comparison of sLOX-1 and hs-TnT Levels Between Patients With and Without Risk Factors

	n	sLOX-1 (pg/ml), median (25–75th percentiles)	P value	hs-TnT (ng/ml), median (25–75th percentiles)	P value
Gender					
Male	160	176.6 (103.3–403.0)	0.153	0.050 (0.010–0.259)	0.586
Female	40	151.9 (100.2–288.3)		0.058 (0.008–0.508)	
Diabetes					
(+)	54	264.9 (167.5–402.5)	0.008	0.080 (0.017–0.252)	0.220
(–)	146	147.3 (96.1–361.3)		0.040 (0.008–0.330)	
Hypertension					
(+)	120	174.6 (108.5–398.2)	0.242	0.055 (0.014–0.278)	0.475
(–)	80	158.3 (91.2–362.9)		0.046 (0.008–0.325)	
Dyslipidemia					
(+)	123	174.5 (106.7–359.4)	0.468	0.058 (0.008–0.270)	0.699
(–)	77	150.7 (94.5–402.8)		0.049 (0.012–0.340)	
Smoking					
(+)	110	218.7 (118.3–402.5)	0.012	0.042 (0.010–0.271)	0.626
(–)	90	130.1 (93.6–317.6)		0.055 (0.010–0.350)	
ACEI/ARB					
(+)	31	156.8 (99.6–362.1)	0.082	0.050 (0.016–0.136)	0.903
(–)	169	281.8 (130.2–434.8)		0.050 (0.009–0.333)	
Statin					
(+)	21	252.0 (169.6–411.3)	0.559	0.035 (0.017–0.141)	0.559
(–)	179	155.5 (99.4–367.5)		0.054 (0.010–0.338)	

sLOX-1, soluble lectin-like oxidized LDL receptor-1; hs-TnT, high-sensitivity troponin T. Other abbreviations see in Table 1.

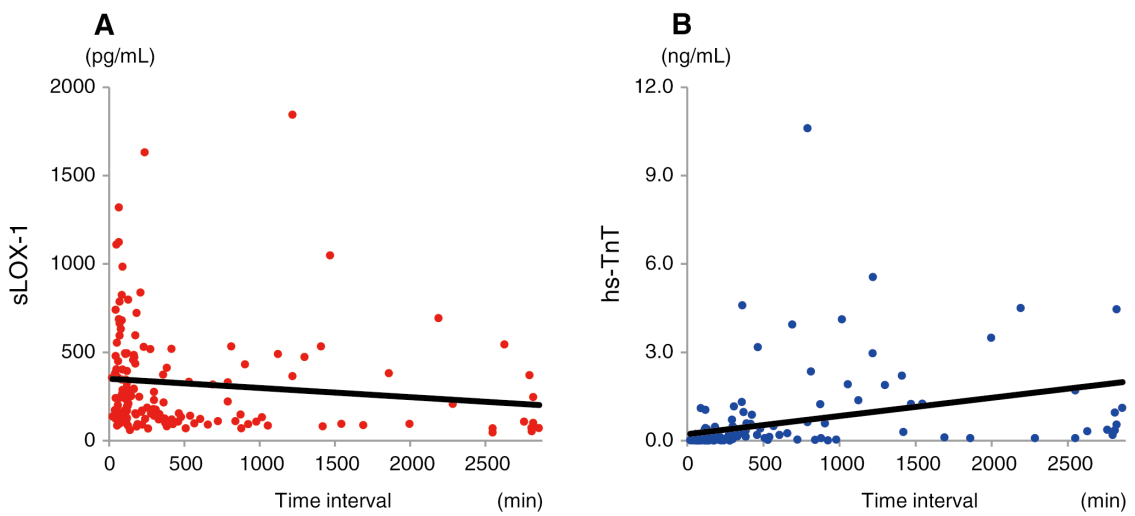

Figure 2. (**A**) Plasma soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) levels inversely correlate with the time interval from the onset of chest symptoms to emergency room arrival (Spearman ρ=–0.313, P<0.001). (**B**) Serum high-sensitivity troponin T (hs-TnT) levels positively correlate with the time interval (Spearman ρ=0.639, P<0.001).

104.1, 67.9 and 128.6 pg/ml for non-ACS, respectively. Plasma sLOX-1 levels were significantly higher in the STEACS (P< 0.001) and NSTEACS (P=0.013) groups than in the non-ACS group, and were significantly more elevated in the STEACS group (P<0.001) than in the NSTEACS group. The respective values of serum hs-TnT for STEACS were 0.060, 0.016 and 0.569 ng/ml, 0.050, 0.020 and 0.217 ng/ml for NSTEACS, and 0.008, 0.004 and 0.050 ng/ml for non-ACS. Serum hs-TnT levels were significantly higher in the STEACS (P<0.001) and NSTEACS (P<0.001) groups than in the non-ACS group. Serum hs-TnT levels were comparable between the STEACS and NSTEACS (P=0.464) patients.

Table 2 shows that plasma sLOX-1, but not serum hs-TnT, levels were significantly higher in patients with diabetes or a smoking habit than those without. Neither hypertension, sex, nor prehospital use of ACEI/ARB or statins significantly

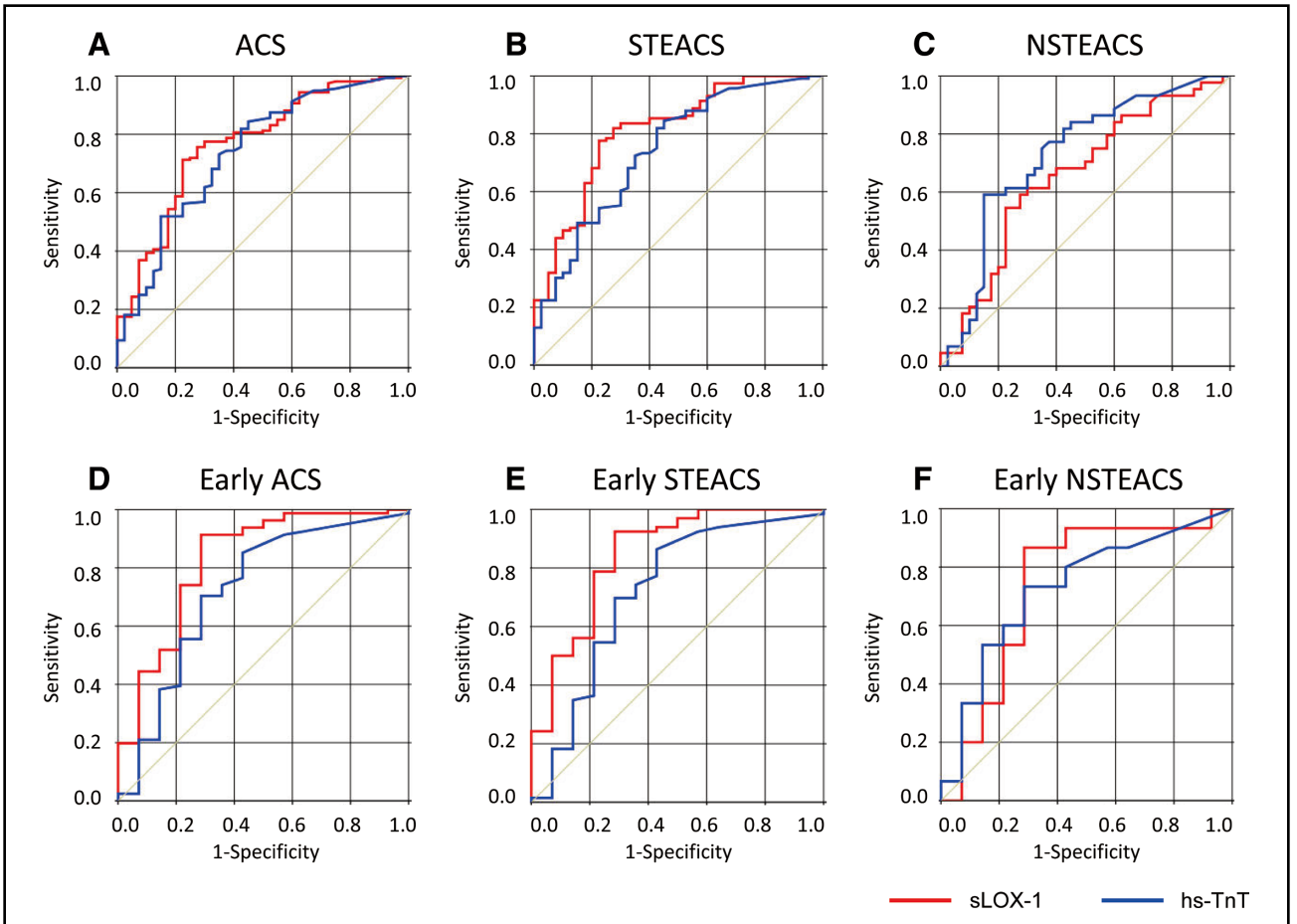

Figure 3. Receiver-operating characteristic curves for diagnostic sensitivity and specificity of soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) and high-sensitivity troponin T (hs-TnT) for diagnosing (**A**) acute coronary syndrome (ACS), (**B**) ST elevation ACS (STEACS), (**C**) non-STE ACS (NSTEACS), (**D**) early ACS, (**E**) early STEACS and (**F**) early NSTEACS. The area under the curve values for sLOX-1 and hs-TnT were 0.769 and 0.739 for ACS, 0.818 and 0.743 for STEACS, and 0.658 and 0.729 for NSTEACS, 0.826 and 0.722 for early ACS, 0.846 and 0.720 for early STEACS, and 0.738 and 0.729 for early NSTEACS, respectively. Early ACS was defined as ACS within 3 h of onset.

affected the plasma sLOX-1 or serum hs-TnT level (**Table 2**). Plasma sLOX-1 levels were significantly and inversely correlated with the time interval from the onset of chest symptoms to ER arrival (**Figure 2A**; Spearman ρ=–0.313, P<0.001). In contrast, serum hs-TnT levels were significantly and positively correlated with this interval (**Figure 2B**; Spearman ρ= 0.639, P<0.001).

Diagnostic Value of sLOX-1 and hs-TnT for ACS

To evaluate the diagnostic accuracy of sLOX-1 and hs-TnT for ACS, STEACS and NSTEACS, their levels were compared between ACS and non-ACS patients, as well as among the STEACS, NSTEACS and non-ACS groups. The ROC curves of sLOX-1 and hs-TnT for detecting ACS, STEACS and NSTEACS using the non-ACS group as a negative reference are shown in **Figures 3A–C** and the diagnostic accuracy determined by these ROC curves is indicated in **Table 3**. The area under the curve (AUC) values of these ROC curves for sLOX-1 and hs-TnT to diagnose ACS were 0.769 and 0.739, respectively. The sensitivity and specificity of sLOX-1 to diagnose ACS were 71.2% and 77.5%, respectively, at an optimal cut-off value of 131.7 pg/ml, and those of hs-TnT were 73.1% and 65.0%, respectively, at an optimal cut-off value of 0.0205 ng/ml.

Comparing the Diagnostic Value of sLOX-1 and hs-TnT for Early Stage of ACS (Within 3 h of the Onset of Symptoms)

The ROC curves of sLOX-1 and hs-TnT for detecting the early stage of ACS, STEACS and NSTEACS, using the early non-ACS group as a negative reference, are shown in **Figures 3D–F** and the diagnostic accuracy determined by these ROC curves is indicated in **Table 3**. The ROC curves indicated that the AUC values for sLOX-1 and hs-TnT to diagnose early ACS were 0.826 and 0.722, respectively. The sensitivity and specificity of sLOX-1 to diagnose early ACS were 91.4% and 71.4%, respectively, at an optimal cut-off value of 116.2 pg/ml, and those for hs-TnT were 70.4% and 71.4%, respectively, at an optimal cut-off value of 0.0109 ng/ml.

Evaluating the Diagnostic Value of sLOX-1 for ACS in Patients With hs-TnT-Negative ACS

ACS patients whose hs-TnT values were less than 0.0205 ng/ml, which was the optimal cut-off value determined from the ROC curve to diagnose ACS (**Figure 3A**), were defined as

Table 3. Diagnostic Value of sLOX-1 and hs-TnT Determined by ROC Curves

	AUC	Cut-off value	Sensitivity (%)	Specificity (%)	P value
To diagnose all ACS					
sLOX-1	0.769	131.7 pg/ml	71.2	77.5	<0.001
hs-TnT	0.739	0.0205 ng/ml	73.1	65.0	<0.001
To diagnose STEACS					
sLOX-1	0.811	131.7 pg/ml	77.6	77.5	<0.001
hs-TnT	0.743	0.0210 ng/ml	72.4	65.0	<0.001
To diagnose NSTEACS					
sLOX-1	0.658	136.1 pg/ml	54.5	77.5	0.013
hs-TnT	0.729	0.0205 ng/ml	75.0	65.0	<0.001
To diagnose all early ACS					
sLOX-1	0.826	116.2 pg/ml	91.4	71.4	<0.001
hs-TnT	0.722	0.0109 ng/ml	70.4	71.4	0.008
To diagnose early STEACS					
sLOX-1	0.846	116.2 pg/ml	92.4	71.4	<0.001
hs-TnT	0.720	0.0114 ng/ml	69.7	71.4	0.010
To diagnose early NSTEACS					
sLOX-1	0.738	119.0 pg/ml	86.7	71.4	0.029
hs-TnT	0.729	0.0109 ng/ml	73.3	71.4	0.036
To diagnose ACS in hs-TnT negative ACS					
sLOX-1	0.869	116.2 pg/ml	90.7	80.8	<0.001
To diagnose STEACS in hs-TnT negative ACS					
sLOX-1	0.898	116.2 pg/ml	90.3	80.8	<0.001
To diagnose NSTEACS in hs-TnT negative ACS					
sLOX-1	0.783	118.1 pg/ml	81.8	80.8	0.007
To diagnose ACS in sLOX-1 negative ACS					
hs-TnT	0.798	0.0520 pg/ml	73.9	83.9	<0.001
To diagnose STEACS in sLOX-1 negative ACS					
hs-TnT	0.825	0.0550 pg/ml	76.9	83.9	<0.001
To diagnose NSTEACS in sLOX-1 negative ACS					
hs-TnT	0.764	0.0205 pg/ml	80.0	71.0	0.002

ROC, receiver-operating characteristics; AUC, area under the curve. Other abbreviations see in Tables 1,2.

the hs-TnT-negative ACS subgroup, and those with hs-TnT more than 0.0205 ng/ml were defined as the hs-TnT-positive ACS subgroup. The characteristics of both these subgroups are summarized in **Table 4**. Sex and the prevalence of coronary risk factors, such as diabetes, hypertension, dyslipidemia and smoking, and prevalence of STEACS, were comparable between these subgroups, except that subjects were younger in the hs-TnT-negative ACS subgroup. Plasma sLOX-1 levels were significantly higher (P=0.034) in the hs-TnT-negative ACS subgroup (median, 25th and 75th percentiles: 269.7, 150.0 and 520.3 pg/ml, respectively) than in the hs-TnT-positive ACS subgroup (median, 25th and 75th percentiles: 199.5, 107.3 and 374.7 pg/ml, respectively). Serum creatinine levels were significantly higher in the hs-TnT-negative ACS subgroup than in the hs-TnT-positive ACS subgroup (P=0.027). Peak CK-MB levels were comparable between these subgroups.

Time intervals from the onset of chest symptoms to ER arrival were significantly shorter (P<0.001) in the hs-TnT-negative ACS subgroup (median, 25th and 75th percentiles: 90, 65 and 136 min, respectively) than in the hs-TnT-positive ACS subgroup (median, 25th and 75th percentiles: 300, 119 and 849 min, respectively). The ROC curves of sLOX-1 for detecting ACS, STEACS and NSTEACS in the respective hs-TnT-negative subgroups, using the hs-TnT-negative non-ACS subgroup as a negative reference, are shown in **Figures 4A–C** and the diagnostic accuracy determined by these ROC curves is indicated in **Table 3**. The AUC values of these ROC curves for sLOX-1 were 0.869, 0.898, and 0.783, to detect ACS, STEACS and NSTEACS, respectively, and the sensitivity and specificity of sLOX-1 to diagnose ACS in the patients with negative hs-TnT ACS were 90.7% and 80.8%, respectively, at the optimal cut-off value of 116.2 pg/ml.

Evaluating the Diagnostic Value of hs-TnT for ACS in Patients With sLOX-1-Negative ACS

Similarly, ACS patients whose sLOX-1 values were less than 131.7 pg/ml, which was the cut-off value determined from the ROC curve to diagnose ACS (**Figure 3A**), were defined as the sLOX-1-negative ACS subgroup, and those with sLOX-1 more than 131.7 pg/ml were defined as the sLOX-1-positive ACS subgroup. Sex and the prevalence of coronary risk factors, such as diabetes, hypertension and dyslipidemia, were comparable between these subgroups, except that smokers were more prevalent in the sLOX-1-positive ACS subgroup. In addition, patients with STEACS were more prevalent in the sLOX-1-positive ACS subgroup. Serum hs-TnT and creatinine levels were comparable between these subgroups. Peak serum CK-MB levels were higher in the positive sLOX-1 ACS subgroup than in the negative sLOX-1 ACS subgroup.

Time intervals from the onset of chest symptoms onset to ER

Table 4. Characteristics of Negative/Positive hs-TnT and sLOX-1 Patients

	hs-TnT-negative ACS (hs-TnT <0.0205) (n=43)	hs-TnT-positive ACS (hs-TnT ≥0.0205) (n=117)	P value	sLOX-1-negative ACS (sLOX-1 <131.7) (n=46)	sLOX-1-positive ACS (sLOX-1 ≥131.7) (n=114)	P value
Male, n (%)	39 (91)	96 (82)	0.182	38 (83)	97 (85)	0.696
Age (years), mean±SD	61±10	66±11	0.014	64±11	65±11	0.836
Risk factors, n (%)						
Diabetes	15 (35)	36 (31)	0.621	11 (24)	40 (35)	0.170
Hypertension	31 (72)	74 (63)	0.296	29 (63)	76 (67)	0.662
Dyslipidemia	29 (67)	74 (63)	0.623	26 (57)	77 (68)	0.188
Smoking	29 (67)	67 (57)	0.244	21 (46)	75 (66)	0.019
STEACS, n (%)	32 (74)	84 (71)	0.742	26 (57)	90 (79)	0.004
sLOX-1 (pg/ml), median (25–75th percentiles)	269.7 (150.0–520.3)	199.5 (107.3–374.7)	0.034	94.4 (80.6–109.5)	327.5 (192.8–493.6)	<0.001
hs-TnT (ng/ml), median (25–75th percentiles)	0.007 (0.005–0.013)	0.178 (0.059–0.580)	<0.001	0.138 (0.032–0.490)	0.050 (0.014–0.320)	0.093
Creatinine (mg/dl), mean±SD	0.81±0.20	0.95±0.38	0.027	0.92±0.45	0.91±0.30	0.769
Peak CK-MB (IU/L), median (25–75th percentiles)	91 (20–183)	88 (34–188)	0.565	47 (13–116)	105 (42–222)	0.001
Interval from the onset of symptoms to ER arrival (min), median (25–75th percentiles)	90 (65–136)	300 (119–849)	<0.001	422 (140–1,056)	130 (76–311)	<0.001
Culprit vessel						
Right coronary artery, n (%)	18 (42)	35 (30)	0.155	14 (30)	39 (35)	0.646
Left anterior descending artery, n (%)	20 (47)	68 (58)	0.191	25 (54)	63 (55)	0.916
Left circumflex artery, n (%)	5 (12)	14 (12)	0.953	7 (15)	12 (11)	0.406
No. of diseased vessels, mean±SD	1.70±0.77	1.79±0.83	0.503	1.63±0.80	1.83±0.81	0.171

Abbreviations see in Tables 1,2.

arrival were significantly shorter (P<0.001) in the sLOX-1-positive ACS subgroup (median, 25th and 75th percentiles: 130, 76 and 311 min, respectively) than in the sLOX-1-negative ACS subgroup (median, 25th and 75th percentiles: 422, 140 and 1,056 min, respectively). The ROC curves of hs-TnT for detecting ACS, STEACS and NSTEACS in the respective sLOX-1-negative subgroups, using the sLOX-1-negative non-ACS subgroups as negative references, are shown in Figures 4D–F and the diagnostic accuracy determined by these ROC curves is indicated in Table 3. The AUC values of these ROC curves for hs-TnT to detect ACS, STEACS and NSTEACS were 0.798, 0.825, and 0.764, respectively, and the sensitivity and specificity of hs-TnT to diagnose ACS in patients with sLOX-1-negative ACS were 73.9% and 83.9%, respectively, at an optimal cut-off value of 0.0520 ng/ml.

Discussion

The universal definition of AMI states that AMI is the diagnosis when blood levels of cardiac biomarkers, preferably troponin I or T, are increased in the clinical setting of acute myocardial ischemia,[9] because cardiac troponins are specific biomarkers of myocardial damage. Highly sensitive assays for cardiac troponins have been developed[7,8] and their sensitivities for diagnosing ACS are increased, so in recent years they have become the gold standard for diagnosing AMI.[9,10] Although some reports have indicated that hs-TnT is a sensitive enough marker of ACS,[8] the sensitivity of hs-TnT to diagnose ACS in the early stage (within 3 h of onset) was demonstrated to be insufficient in another study.[10] In the present study, serum levels of hs-TnT positively correlated with the time interval from the onset of ACS to the collection of blood samples; therefore, the insufficient sensitivity of hs-TnT in the previous study was mainly related to the kinetics of cardiac troponins during the acute stage of ACS. In contrast, the release of sLOX-1, which is regarded as a biomarker of plaque rupture or plaque instability, is expected to precede the release of myocardial damage markers, including cardiac troponins. In fact, the present study showed that sLOX-1 levels were inversely correlated with the time interval from the onset of symptoms to blood sampling. In addition, previous studies reported that sLOX-1 is superior to cardiac troponins, measured by the conventional assay, for diagnosing ACS in the early stage.[13,15–18] In the present study, we directly compared the diagnostic values of sLOX-1 and hs-TnT for ACS, and found that sLOX-1 was superior to hs-TnT during the early stage of ACS (within 3 h of onset).

Although increased assay sensitivity for cardiac troponins has enabled significantly earlier detection of ACS than with the conventional assay,[7,8] that increase has been associated with a decrease in specificity.[11] The specificity of hs-TnT to diagnose ACS appeared to be insufficient in the present study, probably because for the first time non-ACS control subjects, who had chest symptoms and ECG abnormalities, frequently included patients with myocardial damage caused by non-ACS diseases such as CSA, pulmonary thromboembolism, perimyocarditis and takotsubo cardiomyopathy, and presented with elevated serum hs-TnT levels. A recent report indicated that the hs-TnT level was frequently elevated even in patients with nonischemic heart failure.[21] In real clinical settings, it is important to differentiate ACS from the other diseases associated with chest symptoms and ECG abnormalities, but this is often difficult, because both present with elevation of cardiac troponins.

Myoglobin and heart-type fatty acid-binding protein (H-FABP), which are markers of myocardial damage, serve as

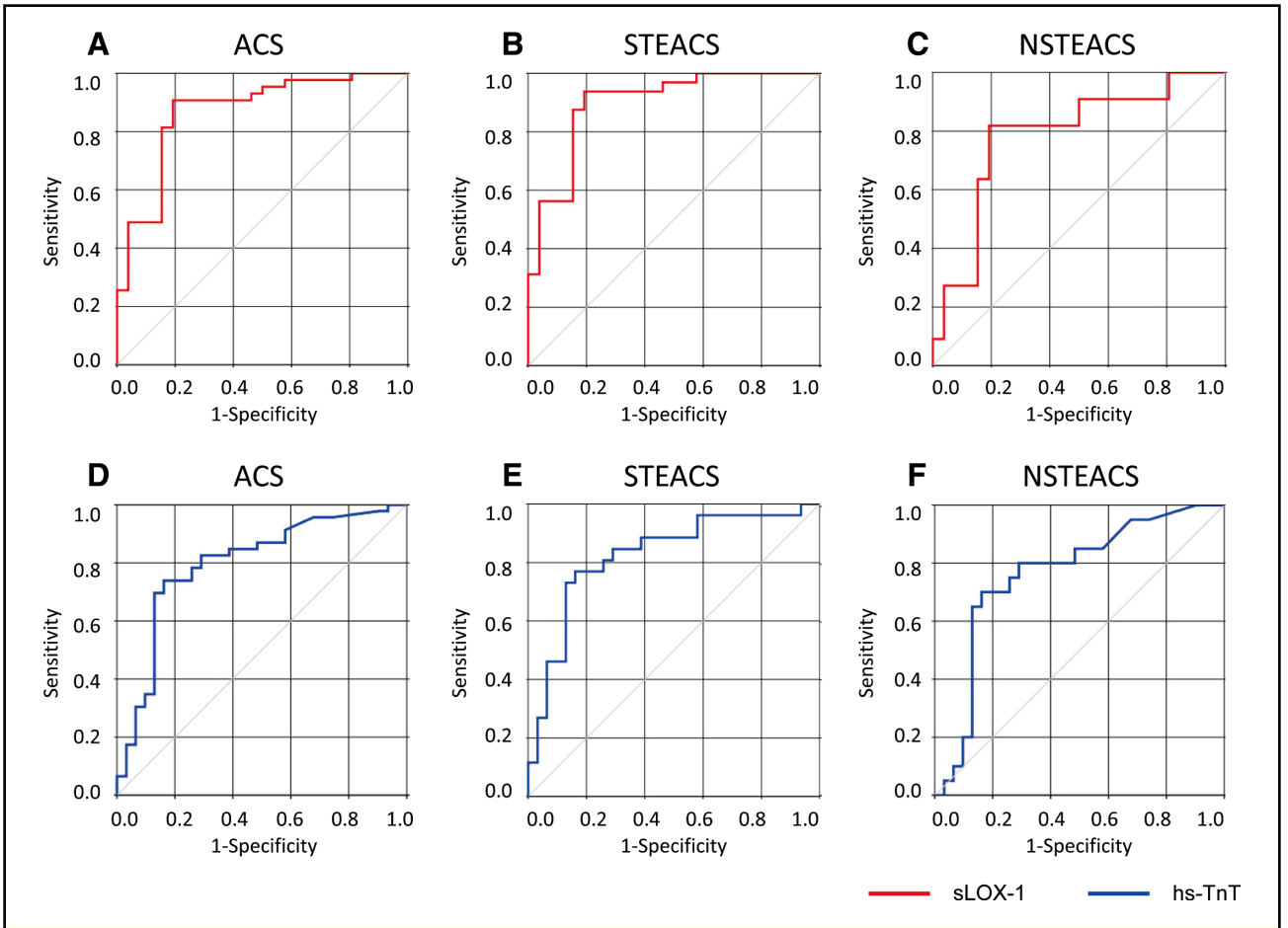

Figure 4. Diagnostic sensitivity and specificity of soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) and high-sensitivity troponin T (hs-TnT) for acute coronary syndrome (ACS) in patients with negative hs-TnT and negative sLOX-1. Receiver-operating characteristic curves for sLOX-1 (**A**–**C**) and hs-TnT (**D**–**F**) for the diagnosis of ACS (**A**, **D**), ST elevation ACS (STEACS; **B**, **E**) and non-STE ACS (NSTEACS; **C**, **F**) in patients with negative hs-TnT (**A**–**C**) and negative sLOX-1 (**D**–**F**) are shown. The area under the curve values of sLOX-1 and hs-TnT were 0.869 and 0.798 for ACS, 0.898 and 0.825 for STEACS, and 0.783 and 0.764 for NSTEACS, respectively. Negative hs-TnT or negative sLOX-1 subgroup was defined as patients with hs-TnT or sLOX-1 values less than 0.0205 ng/ml or 131.7 pg/ml, respectively.

adjunct modalities for diagnosing early-stage AMI because the levels of these biomarkers usually begin to increase at 1 h after onset.[22] Although a combination of cardiac troponins and myoglobin or H-FABP is recommended to improve the diagnostic sensitivity for early-stage AMI,[23] the diagnostic specificity may not be improved because the mechanisms involved in the elevation of cardiac troponins and myoglobin or H-FABP are almost the same. In contrast, the mechanisms involved in the elevation of sLOX-1 levels are different from those of cardiac troponins. Therefore, sLOX-1 is expected to be an adjunctive biomarker that can be combined with troponins to differentiate ACS from other diseases with myocardial damage. In fact, a previous study has shown that sLOX-1 was able to detect ACS in cases in which TnT levels measured by a conventional assay were not increased during the acute stage, with similar sensitivity and specificity to the values determined for the whole population.[19]

In the present study, in which the negative reference was non-ACS patients with similar chest symptoms and ECG abnormalities in the ER, we demonstrated, for the first time, that sLOX-1 was able to detect ACS during the acute stage in cases in which the hs-TnT levels were not increased, with similar sensitivity and specificity to those in the whole population.

LOX-1, a receptor for atherogenic oxidized LDL[12] and the expression of which is induced by factors related to atherogenesis and plaque vulnerability, such as proinflammatory cytokines[24], angiotensin II,[25] high glucose[26] and oxidized LDL,[27] is expressed on the surface of intimal smooth muscle cells[28] and lipid-laden macrophages[28,29] in advanced human atherosclerotic plaques. LOX-1, furthermore, is cleaved at the membrane-proximal extracellular domain by proteases, including a disintegrin and metalloproteinase (ADAM),[13,14] which may also be associated with plaque vulnerability or rupture, thus resulting in release of sLOX-1 into the circulation.[14] In experimental animal models, LOX-1 expression was closely associated with morphological plaque instability and cell apoptosis, as well as with the expression of matrix metalloproteinases and tissue factor, all of which are associated with plaque rupture and thrombus formation.[30–32] Therefore, proteases with enhanced activity in ruptured or rupture-prone vulnerable plaques may be responsible for the cleavage of LOX-1 that is

abundantly expressed in advanced coronary atherosclerotic plaques. Taken together, the evidence points to sLOX-1 being a biomarker of plaque rupture or plaque instability.

The results of the present study clearly demonstrate that plasma levels of sLOX-1 are useful for diagnosing ACS in combination with hs-TnT. Earlier elevation of sLOX-1 allowed us to diagnose ACS in the early stage, which was unable to be diagnosed by hs-TnT alone. On the other hand, neither hs-TnT nor sLOX-1 had sufficient diagnostic specificity for ACS in this cohort, because the negative control subjects included many patients with CSA, arrhythmia, pulmonary thromboembolism, perimyocarditis and takotsubo cardiomyopathy, which also showed elevated hs-TnT levels.

Elevated sLOX-1 levels were also observed in the control subjects, probably because sLOX-1 may also be released into the circulation in certain unknown cases other than coronary plaque rupture or instability. It may be speculated that sLOX-1 is produced by some types of coronary vasospasm, thrombus formation in the pulmonary artery, and reduced cardiac function.[33] Further investigations are necessary to clarify that point.

Plasma sLOX-1 levels were significantly higher in STEACS than in NSTEACS patients, probably because the time intervals from the onset of chest symptoms the ER arrival were significantly shorter in the STEACS than in the NSTEACS group in the present study. In addition, differences in plaque characteristics, such as rupture or erosion, may also have affected the difference in sLOX-1 levels between the STEACS and NSTEACS groups. Plasma sLOX-1 levels were higher in patients with diabetes and a smoking habit than in those without, probably because high glucose levels induce the expression of LOX-1, as previously shown by an in vitro study,[26] and smoking affects plaque vulnerability by inducing oxidative stress and inflammation.[34] Serum peak CK-MB values were higher in patients with a sLOX-1 value ≥131.7 pg/ml in the ER than in those with a value <131.7 pg/ml, which means the sLOX-1 values in the ER probably indicated the severity of ACS. A recent pilot study has shown that sLOX-1 predicted prognosis, such as recurrence or death, in ACS patients.[35] Prognoses of ACS patients should be investigated in larger patient samples in the future. Multidetector row computed tomography[36] and intravascular ultrasound[37] have been used to detect patients with vulnerable plaques. Measuring sLOX-1 may be a less invasive tool for the same purpose. In addition, sLOX-1 could be used to evaluate the therapeutic effects, such as administration of statins[38] or angiotensin II receptor blockers,[39] and body weight reduction,[40] because LOX-1 expression has been shown to be reduced by these interventions. However, in the present study plasma levels of sLOX-1 were comparable between patients with prehospital use of ACEI/ARB or statins and those without. In any case, for early diagnosis of ACS in the ER, it would be desirable for rapid sLOX-1 measurement kits to become commercially available in the future.

In conclusion, using sLOX-1, a biomarker of plaque rupture or vulnerability, or hs-TnT, a biomarker of myocardial damage, alone appears to be insufficient for diagnosing ACS in the real clinical setting. Therefore, the diagnostic accuracy for ACS would be improved by using a combination of sLOX-1 and hs-TnT. However, the limitations of the present study, including its relatively small sample size, indicate that the present findings should be confirmed by multicenter studies with larger sample sizes. Atherosclerotic disease other than coronary artery diseases, such as ischemic stroke,[41] aortic atherosclerosis,[24] and peripheral arteriosclerosis obliterans, probably affect the levels of sLOX-1 because the expression of LOX-1 is not specific for atherosclerosis of coronary arteries. Although some reports have demonstrated a relationship between plaque rupture and sLOX-1 in in-vitro experiments, there have been no reports that indicate that relationship in vivo, so further studies are necessary to clarify the mechanisms of sLOX-1 elevation.

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