厚生労働科学研究費補助金

循環器疾患等総合研究事業（臨床研究実施チームの整備）

心血管疾患のハイリスク患者スクリーニングのための新たな診断システムの構築とその臨床応用（臨床研究実施チームの整備）

平成１７年度総括研究報告書

主任研究者　北　徹

平成１８年３月

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Ⅰ. 研究者構成

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II. 総括研究報告

厚生労働科学研究費補助金
（循環器疾患等総合研究事業（臨床研究実施チームの整備））
総括研究報告書
「心血管疾患のハイリスク患者スクリーニングのための
新たな診断システムの構築とその臨床応用」

主任研究者　名前　北　　徹　　所属　京都大学大学院医学研究科

研究要旨：本臨床研究実施チームのそれぞれの専門性、立場からの協力により、本研究班では、多施設共同で総計 2,000 例の症例を登録し、食事療法、運動療法により、腹囲、血清可溶型酸化 LDL 受容体 LOX-1 値や脂肪細胞分泌因子アディポネクチン値等を含め種々のメタボリックシンドロームに関わる指標が、どのように改善するかを見極める前向き臨床研究の計画を立てた。本計画は京都大学医の倫理委員会の承認を得、まさに研究を開始しようとしている。

A．研究目的

メタボリックシンドロームは心血管イベント発症リスクが増加する疾患として注目を集めている。しかし、我が国における頻度や運動療法等による血清脂質値の改善効果など、不明な点が多く、早急に解明しなければならない。本研究班では、食事療法や運動療法により種々のメタボリックシンドローム関連指標がどのように改善するかを明らかにすることを目的としている。

B．研究方法

上記目的を達成するため、本年度はそのプロトコール作成および、その施行に際して、スムーズに研究が運ぶよう準備を進めてきた。本準備のために、本実施チームは大きな力となった。そして、プロトコールは、2006 年 1 月の研究班会議にて議論され、研究は春よりスタートする。本研究計画「メタボリックシンドローム患者に対する運動療法、食事療法による介入により危険因子をいかに減らせるか」は、2006 年 1 月京都大学医の倫理委員会の承認を得た。

本研究では、京都、東京、千葉、筑波、大阪、山口、福岡、鹿児島の 8 地域における主要医療機関、検診センター及び滋賀における既存のコホートの協力を得て、多施設共同で総計 2,000 例の症例を登録し、食事療法、運動療法（特に、万歩計を持ってもらい毎日の歩数を計測）を指導する。登録症例には、登録時、6ヶ月後、1 年後に、ウエスト周囲径、身長、体重、血圧等を測定し、LDL コレステロール（直接法）、アポリポ蛋白、リポプロテイン(a)、ポリアクリルアミドディスク電気泳動により small dense LDL、ミッドバンドの有無を検討し、アポ B/LDL コレステロール比等、血清脂質プロフィールを測定する。さらに、急性冠症候群で上昇していることを見出した血清可溶型 LOX-1 値 (発表論文 1)や、メタボリックシンドロームとの関連が報告されているアディポネクチン値等を測定する。そして、食事量、運動量と、種々のメタボリックシンドローム関連因子の値の関係（変化率）を明らかにする。

多数の症例を対象とした多施設共同研究であり、質の高い臨床研究を行うため、本臨床

研究実施チームは、その専門分野等より、本研究の円滑な運営、およびデータ解析等に支援、協力を行う。

（倫理面への配慮）
本研究は、京都大学医の倫理委員会の承認を得た。本研究は、ヘルシンキ宣言に乗っ取って、また、個人情報保護法を遵守し、行われる。情報は、個人が特定されない形で保管され、研究報告に際しても、個人が特定されない形で行われる。

C．研究結果：

本年度は、主要研究のプロトコールを構築し、準備を完成させた。2006 年春より開始予定であり、その結果は次年度以降に報告する。

そのため、本報告書では、それぞれのメンバーが実施している研究において得られた結果の中で、本メタボリックシンドロームの研究に参考となるべき結果を記載する。

指導教官、荒井は、厚生科研費による研究である、西暦 2000 年の日本人の血清脂質調査の結果をメタボリックシンドロームの観点よりまとめた（発表論文2）。メタボリックシンドロームの頻度は男性 12.1%、女性 1.7%、全体で 7.8%であった。メタボリックシンドロームの頻度は男性において顕著に多かった。また、男性においては内臓肥満と診断される人が約半数に達した。高脂血症、高血圧、耐糖能異常いずれも男性においてその頻度が高かった。男性においては 30 歳代から増加し始め 40 歳代以降の頻度はほぼ同程度であった。女性においては閉経前に診断基準を満たす人はほとんどいず、閉経後に診断される人がほとんどであった。

ウエスト周囲径が基準以上の人とそうでない人を比較すると男女ともに BMI、収縮期血圧、拡張期血圧、空腹時血糖、総コレステロール、トリグリセリド、LDL コレステロール、HbA1cいずれにおいても基準以上の人のほうが有意に高値を示した。HDL コレステロールについては男女ともに内臓肥満群において有意に低値を示した。インスリンについては男性では内臓肥満群で有意に高値を示したが、女性では有意差を認めなかった。

このように、メタボリックシンドロームの新診断基準による頻度は男性における陽性率が女性に比べ顕著に高いことを報告した。

指導教官、久米は、見出した酸化 LDL 受容体 LOX-1（Nature, 1997）の可溶型血清濃度が急性冠症候群で上昇していることを報告した（発表論文１）。可溶型 LOX-1 濃度は、現在の急性冠症候群の標準的マーカーであるトロポニン T よりも早期に上昇しており、より有用性の高い急性冠症候群診断マーカーになる可能性がある。さらに、慢性期には急性冠症候群の予知因子となる可能性もあり現在、症例数を増やし、種々の疾患でも解析している。本メタボリックシンドロームに関する研究でも、血清可溶型 LOX-1 濃度を測定し、解析していく計画である。

指導教官、堀内は、抗血小板薬服用中の心血管ハイリスク患者を血小板機能や可溶型 LOX-1 や脂肪細胞分泌因子アディポネクチン等を測定しながら予後を追跡するという前向き研究を 2005 年 4 月にスタートさせた。すでに 300 例以上が登録されており、予備解析を開始している。BMI は腹囲によく相関し、特に男性では相関係数 0.86 であった。アスピリンの効果には個人差があり、効果の低い症例で、心血管リスクの増大が

報告されている（発表論文３）。抗血小板薬としてアスピリンのみを服用している患者で、血小板凝集性とBMIの関係を解析したが、相関はなかった。

指導教官、木村および、若手医師、小笹等は2005年、心臓リハビリテーション部門を立ち上げ、心臓病患者に対し、運動療法を積極的に施行している。本メタボリックシンドロームの研究に際しても、その経験、予備データは大きく参考になる。

D．考察

我が国のメタボリックシンドローム患者に対し、食事、運動療法の有効性に関するデータはこれまでのところほとんどない。周辺データや、蓄積してきた臨床研究手法をもとに、研究プロトコールを構築し、倫理委員会の承認も得ることができた。そのプロトコールに則り、早急に研究を開始し、我が国のメタボリックシンドローム患者の治療法に対し、有意義な知見を示したいと考えている。

E．結論

メタボリックシンドロームを対象とし、食事療法や運動療法の効果を判定する研究の準備は整った。本研究を実施し、食事療法、運動療法を基盤にした日本人メタボリックシンドローム治療法に関して基本指針を提供する。

F．健康危険情報

特になし。

G．研究発表

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H. 知的財産権の出願・登録状況
（予定を含む。）
なし

1.特許取得
なし

2.実用新案登録
なし

3.その他
なし

III. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hayashida K, Kume N, Murase T, Minami M, Nakagawa D, Inada T, Tanaka M, Ueda A, Kominami G, Kambara H, Kimura T, Kita T	Serum soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are elevated in acute coronary syndrome: a novel marker for early diagnosis	Circulation	112(6)	812-8	2005
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H. Horiuchi	Recent advance in antiplatelet therapy: mechanisms, evidence and approach to the problems.	Ann. Med	in press		2006

Ⅳ. 研究成果の刊行物・別刷

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

Kazutaka Hayashida, Noriaki Kume, Takatoshi Murase, Manabu Minami, Daisuke Nakagawa, Tsukasa Inada, Masaru Tanaka, Akira Ueda, Goro Kominami, Hirofumi Kambara, Takeshi Kimura and Toru Kita

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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.[1,2] Several diagnostic tests such as echocardiography,[3] radioisotope scintigraphy,[4] and measurement of circulating levels of troponin-T (TnT)[5,6] and the MB isoform of creatine kinase (CPK)[7] 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),[8] oxidized LDL (Ox-LDL),[9] and soluble forms of membrane proteins such as CD40 ligand (CD40L),[10,11] ICAM-1,[12,13] and E-selectin,[12,13] 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,[14] 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 diseas-

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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 proinflammatory 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 cells[30,31] and production of matrix metalloproteinases,[32] which may directly 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 concentrations 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 angiography (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 symptoms (stable angina) and required elective coronary artery revascularization 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 immunonephelometry (Dade Behringer 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 χ^2 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 comparisons. 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 analysis 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

Characteristics	Intact Coronary	Controlled CHD	Ischemic CHD	Non-ACS CAG	ACS
Patients, n	52	122	173	Subtotal, 347	80
Age (mean±SD), y	66±9	66±10	67±9	67±10	64±12
Male sex, n (%)	32 (62)	89 (73)	128 (74)	249 (67)	59 (74)
Risk factors, n (%)					
Hypertension	22 (42)	65 (53)	82 (47)	168 (48)	30 (38)
Diabetes	8 (15)†	43 (35)	63 (36)	114 (33)	26 (33)
Smoking	19 (37)	61 (50)	57 (33)‡	136 (39)*	43 (54)*
Hypercholesterolemia	14 (27)	47 (39)	84 (49)§	144 (41)	27 (34)
Lipid profile (mean±SD), mg/dL					
LDL cholesterol	122±38	125±35	121±35	121±36	122±35
HDL cholesterol	50±16	45±13	45±14	46±14¶	41±11¶#
Triglycerides	137±103	141±63	132±52	136±68	140±75
hs-CRP (mean±SD), ng/mL	3.10±0.75	3.09±0.65	3.11±0.87	3.10±0.78¶	3.41±0.87‖¶

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 χ^2 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 intact coronary, controlled CHD, and ischemic CHD.

¶$P<0.001$ for comparison between non-ACS CAG and ACS with t test.

#$P<0.05$ for comparison with intact coronary.

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

Characteristics	Noncardiac Chronic Illness	Noncardiac Acute Illness	Non-ACS CAG	Combined All Non-ACS	ACS
Patients, n	60	34	347	Subtotal, 441	80
Age (mean±SD, y	67±16	54+18†	67±10	66±13	64±12
Male sex, n (%)	18 (30)‖	21 (62)	249 (67)	288 (65)	59 (74)
Risk factors, n (%)					
Hypertension	16 (27)	9 (26)	168 (48)§	193 (44)	30 (38)
Diabetes	5 (8)§	2 (6)§	114 (33)	121 (27)	26 (33)
Smoking	9 (15)‖	13 (38)	136 (39)	158 (36)*	43 (54)*
Hypercholesterolemia	25 (42)	6 (18)†	144 (41)	175 (40)	27 (34)
Lipid profile (mean±SD), mg/dL					
LDL cholesterol	127±30	101±37†	121±36	122±36	122±35
HDL cholesterol	59±18	56±17	46±14	49±16¶	41±11‡¶
Triglycerides	147±106	120±168	136±68	136±86	140±75
hs-CRP (mean±SD), ng/mL	3.14±0.58	4.17±1.02†	3.10±0.78	3.19±0.83	3.41±0.87#

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 χ^2 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 chronic illness, non-ACS CAG, and ACS.

¶$P<0.001$ for comparison between combined all non-ACS and ACS with t test.

#$P<0.05$ for comparison with non-ACS CAG.

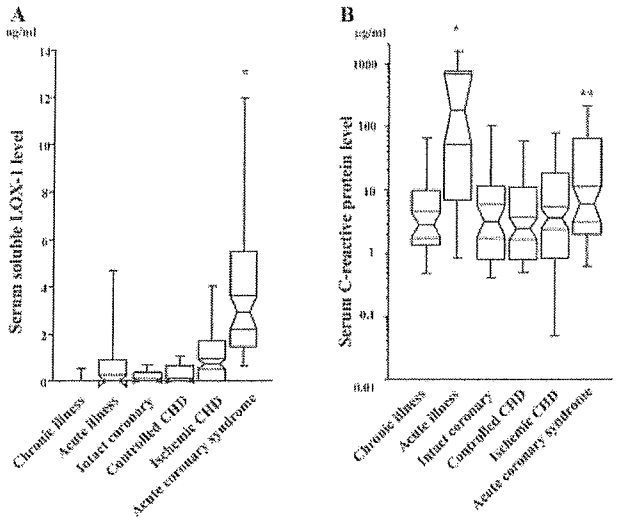

Figure 1. Serum sLOX-1 and hs-CRP levels. In 427 consecutive patients who underwent CAG, consisting of 80 with ACS, 173 with symptomatic CHD (ischemic CHD), 122 with coronary atherosclerosis without ischemia (controlled CHD), and 52 without apparent coronary atherosclerosis (intact coronary) plus 34 with noncardiac acute illness (acute illness) and 60 patients with noncardiac chronic illness (chronic illness), serum LOX-1 (A) and hs-CRP (B) levels were determined and are indicated in box plots. Center horizontal lines indicate median values; inner trapezoidal boxes, 95% CIs for medians; upper and lower edges of outer boxes, 25th and 75th percentiles; and lower and upper bars, 10th and 90th percentiles. *Statistically significant differences among the 6 groups by Kruskal-Wallis test with Dunn's test (A) and 1-way ANOVA with Tukey-Kramer test (B) ($P<0.05$). **Significant differences among 4 CAG groups by 1-way ANOVA with Tukey-Kramer test ($P<0.05$).

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 ($\chi^2=88.2$, $P<0.001$), 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).

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

	sLOX-1	hs-CRP	TnT
Non-ACS CAG (n=347)			
Positive, n	86	91	...
Specificity	75	74	...
All ACS (n=80)			
Positive, n	65	36	54
χ^2	88.2	12	...
P	<0.001	<0.001	...
Sensitivity	81	45	68
NQ-ACS (n=23)			
Positive, n	21	9	11
χ^2	43.2	1.7	...
P	<0.001	0.22	...
Sensitivity	91	39	48
ACS with TnT negative at the time of visit (n=24)			
Positive, n	20	3	...
χ^2	37.8	3.1	...
P	<0.001	0.2	...
Sensitivity	83	13	...

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. χ^2 was determined by the Yates continuity-corrected χ^2 test, and probability values were obtained by comparison with non-ACS patients.

Significant inverse correlation was found between sLOX-1 and HDL cholesterol levels (Spearman's $\rho=-0.17$; $P<0.01$). However, no significant correlation was found between sLOX-1 and either LDL cholesterol (Spearman's $\rho=-0.02$; $P=0.68$) or triglyceride (Spearman's $\rho=-0.01$, $P=0.89$) 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 $R^2=0.263$) 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 $\rho=0.01$ 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

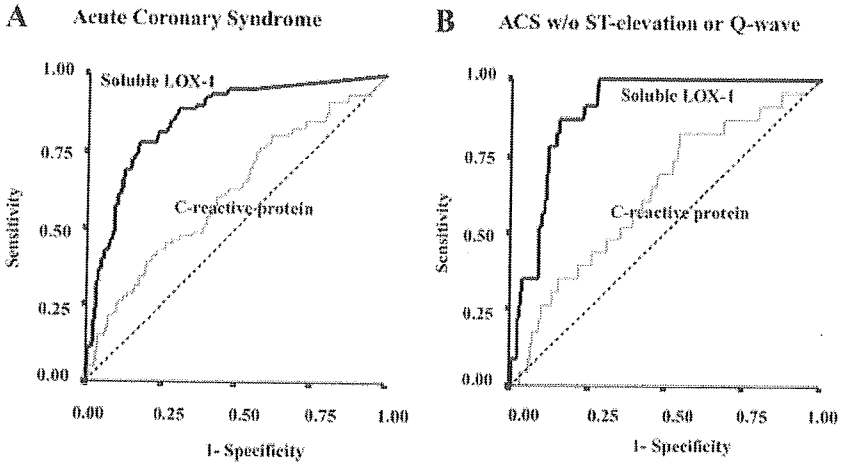

Figure 2. ROC curves of sLOX-1 and hs-CRP for diagnosis of ACS (A) and ACS without ST elevation or abnormal Q-waves (NQ-ACS; B) among consecutive patients undergoing coronary angiography. True-positive fraction (sensitivity as *y* axis) was plotted vs false-positive fraction (1−specificity as *x* axis)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

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 μg/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 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 ($P<0.01$).[37,38] 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

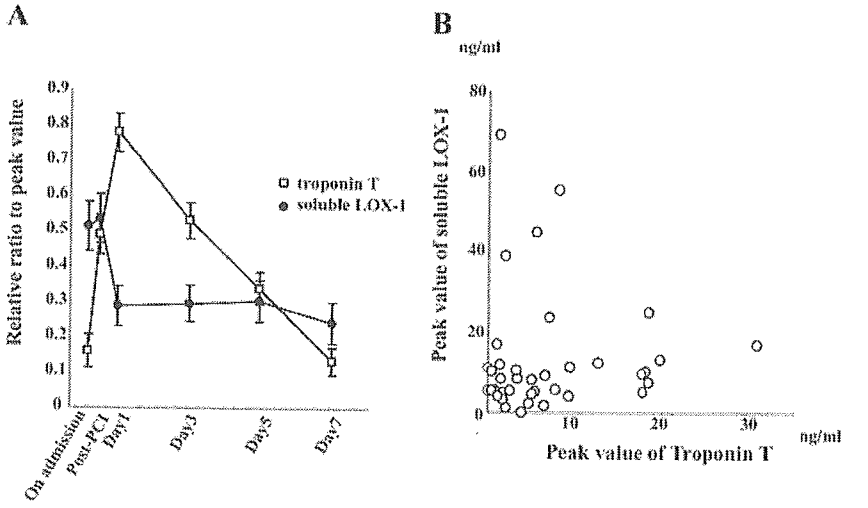

Figure 3. Time-dependent changes in sLOX-1 and TnT levels after onset of ACS (A) and comparison between peak values of sLOX-1 and TnT (B). Blood samples were collected on admission, immediately after PCI (post-PCI), and on days 1, 3, 5, and 7 from 40 ACS patients undergoing emergency PCI. Relative ratios (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,[33] as is the case for CD40L.[14] However, LOX-1 can also bind activated platelets[40]; 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,[41] 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[29] and remarkably inducible by proinflammatory stimuli,[23,25,26] 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,[42] 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.[9,43] 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 CPK, 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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