

# Malondialdehyde-Modified LDL as a Marker of Acute Coronary Syndromes

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**I**SCHEMIC INJURY OF ENDOTHELIUM IS associated with prostaglandin synthesis and platelet adhesion and aggregation, which may be associated with the release of aldehydes that substitute the lysine residues in the apolipoprotein B-100 moiety of low-density lipoprotein (LDL) in the absence of lipid peroxidation.<sup>1,2</sup> This type of oxidatively modified LDL is referred to as malondialdehyde (MDA)-modified LDL.<sup>3</sup> Plasma levels of MDA-modified LDL are increased in patients with acute coronary syndromes, ie, unstable angina or acute myocardial infarction (AMI).<sup>3,4</sup> Previously, C-reactive protein<sup>5</sup> and cardiac troponins<sup>6-9</sup> have been proposed as diagnostic markers of acute coronary syndromes. In this study, we compared the usefulness of MDA-modified LDL as a marker of acute coronary syndromes with that of C-reactive protein and troponin I.

## METHODS

A total of 168 patients were studied, including 104 consecutive patients admitted to the emergency department of the University Hospital of Leuven, Belgium, because of an acute coronary syndrome and 64 patients with stable, angiographically documented coronary artery disease (CAD) without clinical signs of ischemia. Patients with stable CAD were treated with aspirin,  $\beta$ -blockers, nitrates, and, in a great majority, angiotensin-converting enzyme inhibitors. Patients with acute coronary syndromes had ischemic chest discomfort with transient ST-segment elevation

**Context** Release of circulating malondialdehyde (MDA)-modified low-density lipoprotein (LDL) may reflect endothelial injury or plaque instability.

**Objective** To determine the usefulness of MDA-modified LDL for identifying patients with unstable angina and acute myocardial infarction (AMI).

**Design** Blinded comparison of MDA-modified LDL, C-reactive protein, and troponin I followed by multiple receiver operating curve analysis.

**Setting** University hospital.

**Participants** A total of 104 consecutive patients with acute coronary syndromes (42 with unstable angina and 62 with AMI), and 64 patients with stable coronary artery disease (CAD) without evidence of ischemia.

**Main Outcome Measures** Ability of MDA-modified LDL, C-reactive protein, and troponin I to discriminate patients with stable CAD, unstable angina, or AMI.

**Results** Malondialdehyde-modified LDL ( $\chi^2 = 10.2$ ;  $P = .001$ ), but not troponin I or C-reactive protein, discriminated between stable CAD and unstable angina. Troponin I ( $\chi^2 = 14.5$ ;  $P < .001$ ), but not MDA-modified LDL or C-reactive protein, discriminated between unstable angina and AMI. Both MDA-modified LDL and troponin I ( $\chi^2 = 14.5$ ;  $P < .001$  and  $\chi^2 = 5.3$ ;  $P = .02$ , respectively) but not C-reactive protein discriminated between stable CAD and AMI. The sensitivity of MDA-modified LDL was 95% for unstable angina and 95% for AMI, with a specificity of 95%. Values for troponin I were 38% and 90%, respectively, with a specificity of 95%. The combination of MDA-modified LDL and troponin I had a sensitivity of 98% for unstable angina and 100% for AMI, with a specificity of 99%.

**Conclusion** The combination of MDA-modified LDL, which may reflect endothelial injury or plaque instability, and troponin I, which reflects myocardial cell injury, allows better discrimination between stable CAD and acute coronary syndromes than troponin I alone.

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or persistent ST-segment depression of more than 0.5 mm, or T-wave inversion of more than 1 mm. Blood samples for MDA-modified LDL assay from patients with acute coronary syndromes were collected at admission, within 6-8 hours of onset of chest pain, and before therapy was started. Blood samples for total creatine kinase (CK) and for CK isoenzyme MB mass assay were obtained at entry, 6 to 8 and then 12 to 16 hours after admission. Enzyme evidence of AMI was defined as CK-MB elevation above the normal range and at least 3% of total CK. All patients with acute coronary syndromes received oral aspirin, intravenous nitrates, and heparin for 48 to 72 hours. Most of these

patients underwent coronary angiography and revascularization within the first 3 to 5 days after hospital admission. Blood samples were collected in

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the fasting state in patients with stable CAD.

Venous blood samples were collected in 0.1 volume of 0.1 mol/L of citrate, containing 1 mmol/L of EDTA, 20  $\mu$ mol/L of vitamin E, 10  $\mu$ mol/L of butylated hydroxytoluene, 20  $\mu$ mol/L of dipyrindamole, and 15 mmol/L of theophylline. Blood samples were centrifuged at 3000g for 15 minutes at room temperature within 1 hour after collection and stored at  $-30^{\circ}\text{C}$  until assays were performed.<sup>3,4,10</sup>

A monoclonal antibody 1H11-based competition enzyme-linked immunosorbent assay (ELISA) was used for the quantitation of MDA-modified LDL in plasma.<sup>3,4</sup> The  $C_{50}$  values, ie, concentrations that are required to obtain 50% inhibition of antibody binding to immobilized in vitro MDA-modified LDL in the ELISA, are 0.65 mmol/L (25 mg/dL) for native LDL, 0.001 mmol/L (0.05 mg/dL) for MDA-modified LDL with at least 60 aldehyde-substituted lysines per apolipoprotein B-100, and 0.06 mmol/L (2.5 mg/dL) for oxidized LDL with fragmented apolipoprotein B-100 moiety that was generated via copper ion-induced lipid peroxidation. Intra-assay and interassay coefficients of variation were 12% and 15%, respectively. When in vitro MDA-modified LDL was added to control human plasma at a final concentration of 0.006 mmol/L (0.25 mg/dL), recovery was 95%. Ten plasma samples from AMI patients were thawed, assayed, and frozen again. The MDA-modified LDL levels (SDs) in these samples were 0.04 (0.003) mmol/L (1.4 [0.1] mg/dL) after the first thawing, 0.03 (0.005) mmol/L (1.3 [0.2] mg/dL) after the second thawing, and 0.04 (0.005) mmol/L (1.5 [0.2] mg/dL) after the third thawing. Levels of MDA-modified LDL were measured blindly at the University of Leuven's Center for Molecular and Vascular Biology, where the test was developed.

Total and high-density lipoprotein (HDL) cholesterol and triglyceride levels were measured by enzymatic methods (Boehringer Mannheim, Mannheim, Germany). Low-density li-

poprotein cholesterol levels were calculated with the Friedewald formula. Creatine kinase activity and CK-MB mass were measured using commercially available assays (Boehringer Mannheim and Abbott Laboratories, North Chicago, Ill, respectively). C-reactive protein levels were measured with a commercial immunoassay (Boehringer Mannheim). All measurements were performed at the clinical laboratory of the University Hospital in Leuven. Cardiac troponin I levels were measured on a Beckman ACCESS immunoanalyzer (Analisis, Gent, Belgium) using commercially available monoclonal antibodies (Sanofi, Toulouse, France). These measurements were performed in the laboratories of Analisis.

Plasma levels of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, MDA-modified LDL, C-reactive protein, and troponin I were compared in patients with stable CAD and those with acute coronary syndromes by nonparametric Kruskal-Wallis test followed by the Dunnett multiple-comparison test. Discontinuous parameters were compared by  $\chi^2$  analy-

sis. Logistic regression models were used to determine the associations between acute coronary syndromes and C-reactive protein, cardiac troponin I, and MDA-modified LDL. For continuous variables, cubic spline functions were used to model the relationship between covariates and response. Multiple receiver operating characteristic (ROC) curve analysis<sup>11</sup> was performed to determine if C-reactive protein, troponin I, or MDA-modified LDL could discriminate among stable CAD, unstable angina, and AMI. All analyses were performed with SPlus, Version 4.0 (Mathsoft Inc, Cambridge, Mass) and SAS/STAT, Version 6.12 (SAS Institute Inc, Cary, NC).  $P < .05$  was considered statistically significant.

## RESULTS

Of the 104 patients with acute coronary syndromes, 62 had elevated CK-MB levels indicative of AMI. In 42 patients, no CK-MB elevations were found, and these patients were classified as having unstable angina pectoris. Patients with stable CAD and those with acute coronary syndromes were

**Table 1.** Clinical and Laboratory Characteristics of Patients With Stable CAD (n=64) and Acute Coronary Syndromes (n=104)\*

Characteristics	Stable CAD (n = 64)	Unstable Angina (n = 42)	AMI (n = 62)
Age, y	65 (10)	72 (12)	63 (11)
Sex, male/female	53/11	28/14	41/21
Total cholesterol			
mmol/L	4.58 (0.91)	4.50 (0.96)	4.53 (0.96)
mg/dL	177 (35)	174 (37)	175 (37)
LDL-C			
mmol/L	2.97 (0.78)	2.82 (0.85)	2.87 (0.83)
mg/dL	115 (30)	109 (33)	111 (32)
HDL-C			
mmol/L	0.98 (0.34)	1.16 (0.41)	0.98 (0.25)
mg/dL	38 (13)	45 (16)	38 (9.7)
Triglycerides			
mmol/L	1.39 (0.52)	1.13 (0.62)	1.42 (0.64)
mg/dL	12.3 (46)	100 (55)	126 (57)
C-reactive protein, mg/dL	5.30 (6.89)	12.48 (23.50)	18.41 (31.58)†
Troponin I, $\mu$ g/L	0.035 (0.095)	0.19 (0.44)†	0.90 (1.68)†
MDA-modified LDL			
mmol/L	0.01 (0.005)	0.03 (0.01)†	0.03 (0.01)†
mg/dL	0.46 (0.20)	1.33 (0.49)†	1.19 (0.49)†

\*CAD indicates coronary artery disease; AMI, acute myocardial infarction; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and MDA, malondialdehyde. Data are mean (SD) unless otherwise noted. Continuous parameters were compared by nonparametric Kruskal-Wallis test followed by Dunnett multiple comparison test, whereas male-female ratios were compared by  $\chi^2$  test.  
† $P < .001$  vs stable CAD.

similar with respect to age and sex as well as serum levels of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides (TABLE 1).

Compared with patients with stable CAD, C-reactive protein levels were 2.4-fold higher in patients with unstable angina ( $P = .02$ ) and 3.5-fold higher in patients with AMI ( $P < .001$ ). Plasma levels of C-reactive protein were somewhat higher ( $P = .02$ ) in patients with AMI than those with unstable angina (Table 1). Compared with patients with stable CAD, troponin I levels were 5.4-fold higher in patients with unstable angina ( $P < .001$ ) and 26-fold higher in patients with AMI ( $P < .001$ ). Plasma troponin I levels were significantly higher in patients with AMI than those with unstable angina ( $P < .001$ ) (Table 1). Compared with patients with stable CAD, plasma levels of MDA-modified LDL were 2.9-fold higher in patients with unstable angina ( $P < .001$ ) and 2.6-fold higher in patients with AMI ( $P < .001$ ). Plasma levels of MDA-modified LDL were similar in patients with unstable angina and those with AMI ( $P = .09$ ).

Univariate logistic regression analysis revealed an association of clinically

diagnosed acute coronary syndromes with C-reactive protein ( $\chi^2 = 21$ ;  $P < .001$ ), troponin I ( $\chi^2 = 25$ ;  $P < .001$ ) and MDA-modified LDL ( $\chi^2 = 19$ ;  $P < .001$ ). C-reactive protein correlated with MDA-modified LDL ( $r = 0.32$ ;  $P = .01$ ) and troponin I ( $r = 0.59$ ;  $P < .001$ ); troponin I correlated with C-reactive protein and MDA-modified LDL ( $r = 0.44$ ;  $P < .001$ ). Malondialdehyde-modified LDL correlated with both troponin I and C-reactive protein.

Multiple ROC analysis revealed that MDA-modified LDL ( $\chi^2 = 10.2$ ;  $P = .001$ ), but not troponin I and C-reactive protein, discriminated between stable CAD and unstable angina. In contrast, troponin I ( $\chi^2 = 14.5$ ;  $P < .001$ ), but neither MDA-modified LDL nor C-reactive protein, discriminated between unstable angina and AMI. Both MDA-modified LDL ( $\chi^2 = 14.5$ ;  $P < .001$ ) and troponin I ( $\chi^2 = 5.3$ ;  $P = .02$ ), but not C-reactive protein, discriminated between stable CAD and AMI.

As shown in TABLE 2, at a cutoff value of 10 mg/dL (the value exceeding the 95th percentile of distribution in patients with stable angina), the sensitivity of C-reactive protein was 19% for unstable angina and 42% for AMI,

whereas the specificity was 95%. At a cutoff value of 0.05  $\mu\text{g/L}$  (the value exceeding the 95th percentile of distribution in patients with stable angina), the sensitivity of troponin I was 38% for unstable angina and 90% for AMI, whereas the specificity was 95%. At a cutoff value of 0.02 mmol/L (0.70 mg/dL; the value exceeding the 95th percentile of distribution in patients with stable angina), the sensitivity of MDA-modified LDL was 95% for unstable angina and 95% for AMI, and the specificity was 95%.

The study cohort included 98 patients (35 with stable CAD, 18 with unstable angina, and 45 with AMI) who have been described elsewhere<sup>4</sup> and 70 new patients (29 with stable CAD, 24 with unstable angina, and 17 with AMI). The age; sex; serum levels of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides; and levels of C-reactive protein, troponin I, and MDA-modified LDL of patients with stable angina and acute coronary syndromes were similar in the previously reported subjects and the overall study cohort, as were the sensitivities for troponin I and MDA-modified LDL for unstable angina and AMI.

**Table 2.** Ratios of Positive vs Negative Test Results for C-Reactive Protein, Troponin I, and MDA-Modified LDL in Patients With Stable CAD and Acute Coronary Syndromes\*

Parameters	Stable CAD Ratio	Unstable Angina			AMI			Acute Coronary Syndromes		
		Ratio	$\chi^2$	P Value	Ratio	$\chi^2$	P Value	Ratio	$\chi^2$	P Value
C-reactive protein	3:61	8:34	4.2	.04	26:36	23	.001	34:70	17	<.001
Troponin I	3:61	16:26	17	<.001	56:6	89	.001	72:32	64	<.001
MDA-modified LDL	3:61	40:2	83	<.001	59:3	100	<.001	99:5	132	<.001

\*MDA indicates malondialdehyde; LDL, low-density lipoprotein; CAD, coronary artery disease; and AMI, acute myocardial infarction. Cutoff values were 10 mg/dL for C-reactive protein, 0.05  $\mu\text{g/L}$  for troponin I, and 0.02 mmol/L (0.70 mg/dL) for MDA-modified LDL. All these values exceeded the 95th percentile of distribution in individuals with stable CAD. The ratios between positive and negative results are represented;  $\chi^2$  values, determined by the Yates continuity corrected  $\chi^2$  test, and P values were obtained by comparison with stable CAD patients.

**Table 3.** Sensitivity of MDA-Modified LDL and Troponin I in Patients With Stable CAD or Acute Coronary Syndromes\*

	MDA-Modified LDL $\geq 0.02$ mmol/L (0.70 mg/dL) and Troponin I $\geq 0.05$ $\mu\text{g/L}$	MDA-Modified LDL $\geq 0.02$ mmol/L (0.70 mg/dL) and Troponin I $< 0.05$ $\mu\text{g/L}$	MDA-Modified LDL $< 0.02$ mmol/L (0.70 mg/dL) and Troponin I $\geq 0.05$ $\mu\text{g/L}$	MDA-Modified LDL $< 0.02$ mmol/L (0.70 mg/dL) and Troponin I $< 0.05$ $\mu\text{g/L}$	$\chi^2$	P Value
Stable CAD	1 (1.6)	3 (4.7)	3 (4.7)	57 (89)	...	...
Unstable angina	13 (31)	27 (64)	1 (2.5)	1 (2.5)	73	<.001
AMI	53 (85.5)	6 (9.7)	3 (4.8)	0 (0)	97	<.001
Acute coronary syndromes	66 (63.4)	33 (31.7)	4 (3.8)	1 (0.96)	132	<.001

\*Data in parentheses are percentage of patients. MDA indicates malondialdehyde; LDL, low-density lipoprotein; CAD, coronary artery disease; and AMI, acute myocardial infarction. Cutoff values are the same as in Table 2. The Yates continuity corrected  $\chi^2$  test was performed to compare the number of positive test results (either for both MDA-modified LDL and troponin I or for MDA-modified LDL or troponin I alone) in patients with stable CAD and acute coronary syndromes.

As shown in TABLE 3, when MDA-modified LDL and troponin I were combined, the sensitivity was 98% for unstable angina compared with 95% when MDA-modified LDL was used alone and 38% when troponin I was used alone. When MDA-modified LDL and troponin I were combined, the sensitivity was 100% for AMI compared with 95% for MDA-modified LDL alone and 90% for troponin I alone. The specificity increased from 95% for MDA-modified LDL or troponin I alone to 99% for their combination.

## COMMENT

We compared the usefulness of MDA-modified LDL for the diagnosis of unstable angina and AMI with C-reactive protein and cardiac troponins, which have been proposed as diagnostic markers for acute coronary syndromes.<sup>5-9</sup> Mach et al<sup>12</sup> reported increased levels of C-reactive protein in only 5% of patients with unstable angina. Hamm et al<sup>13</sup> found positive troponin I test results in 36% of patients with unstable angina, whereas Galvani et al<sup>14</sup> found positive troponin I test results in only 24% of patients with unstable angina. In the present study, the rather low diagnostic values of C-reactive protein and troponin I for unstable angina were confirmed: their sensitivities were 19% and 38%, respectively. However, troponin I was found to be a clinically useful marker for AMI—the sensitivity was

90% compared with 100% reported by Hamm et al.<sup>13</sup>

Because an increase of troponin I depends on myocardial cell injury, it is logical to assume that troponin I is a better marker for AMI than for unstable angina. We hypothesized that a parameter that has been associated with ischemic injury of endothelium, plaque instability, or both, which precede cardiac necrosis, would be a more sensitive marker for unstable angina than troponin I. Multiple ROC analyses showed that MDA-modified LDL better discriminated between stable CAD and unstable angina than troponin I. The sensitivity of MDA-modified LDL for unstable angina was 95% compared with 38% for troponin I. The similar sensitivities of MDA-modified LDL for unstable angina and AMI suggest that it may be a clinically useful marker of acute coronary syndromes irrespective of the occurrence of myocardial cell injury.

Endothelial injury in association with hypoxia may result in the activation of not only the cyclooxygenase-dependent pathway of prostaglandin synthesis in endothelial cells<sup>15</sup> but also in increased production of F<sub>2α</sub>-isoprostanes, noncyclooxygenase-derived prostaglandin-like compounds<sup>1,16</sup> that are strong inducers of platelet activation. Plaque instability is associated with increased platelet adhesion and activation. Activated platelets may then produce large amounts of aldehydes, further enhanc-

ing the modification of LDL. The association of unstable angina and AMI with plasma levels of MDA-modified LDL supports the hypothesis that the generation of MDA-modified LDL is associated with ischemic injury or plaque instability rather than with the extent of coronary atherosclerosis. The low reactivity of the monoclonal antibody 1H11 with nonthrombotic atherosclerotic plaques, in contrast with the high reactivity with unstable plaques, suggests that MDA-modified LDL, in contrast with oxidized LDL, is not released continuously from atherosclerotic plaques but is generated in unstable plaque.<sup>4</sup>

In conclusion, this study shows that the combination of MDA-modified LDL, which may reflect endothelial injury or plaque instability,<sup>3,4</sup> and troponin I, which reflects myocardial cell injury,<sup>13</sup> allows a more sensitive and specific discrimination between stable CAD and acute coronary syndromes than troponin I alone. Further evaluation of the prognostic value of MDA-modified LDL for AMI in patients with unstable angina requires large-scale prospective studies.

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