Circulating Oxidized LDL Is a Useful Marker for Identifying Patients With Coronary Artery Disease

Paul Holvoet, Ann Mertens, Peter Verhamme, Kris Bogaerts, Guy Beyens, Raymond Verhaeghe, Désiré Collen, Erik Muls, Frans Van de Werf

Abstract—Our aim was to determine the usefulness of circulating oxidized low density lipoprotein (LDL) in the identification of patients with coronary artery disease (CAD). A total of 304 subjects were studied: 178 patients with angiographically proven CAD and 126 age-matched subjects without clinical evidence of cardiovascular disease. The Global Risk Assessment Score (GRAS) was calculated on the basis of age, total and high density lipoprotein cholesterol, blood pressure, diabetes mellitus, and smoking. Levels of circulating oxidized LDL were measured in a monoclonal antibody 4E6–based competition ELISA. Compared with control subjects, CAD patients had higher levels of circulating oxidized LDL ($P<0.001$) and a higher GRAS ($P<0.001$). The sensitivity for CAD was 76% for circulating oxidized LDL (55% for men and 81% for women) compared with 20% (24% for men and 12% for women) for GRAS, with a specificity of 90%. Logistic regression analysis revealed that the predictive value of oxidized LDL was additive to that of GRAS ($P<0.001$). Ninety-four percent of the subjects with high (exceeding the 90th percentile of distribution in control subjects) circulating oxidized LDL and high GRAS had CAD (94% of the men and 100% of the women). Thus, circulating oxidized LDL is a sensitive marker of CAD. Addition of oxidized LDL to the established risk factors may improve cardiovascular risk prediction. **(*Arterioscler Thromb Vasc Biol*. 2001;21:844-848.)**

Key Words: atherosclerosis ■ coronary artery disease ■ diagnosis ■ lipoproteins

Major independent risk factors for coronary artery disease (CAD) are advancing age, elevated blood pressure, elevated serum total and LDL cholesterol levels, low serum HDL cholesterol level, diabetes mellitus, and cigarette smoking.[1–3] The Framingham Heart Study[1] has elucidated the quantitative relationship between these risk factors and CAD. It has shown that the major risk factors are additive in predictive power. Accordingly, the total risk of a person can be estimated by a summing of the risk imparted by each of the major risk factors. Recently, the American Heart Association and the American College of Cardiology issued a scientific statement that assessed the Global Risk Assessment Scoring (GRAS) as a guide to primary prevention.[4] GRAS is based on age, total and HDL cholesterol levels, systolic blood pressure, diabetes mellitus, and smoking. Predisposing factors such as obesity, physical inactivity, and family history of premature CAD are not included in GRAS.

Elevated levels of oxidized LDL have previously been detected in the plasma of CAD patients.[5–7] Therefore, we determined the predictive value of circulating oxidized LDL for CAD. Logistic regression analysis was used to determine whether the predictive value of circulating oxidized LDL was additive to that of GRAS. Finally, the correlation between circulating oxidized LDL and major cardiovascular risk factors in subjects without clinical evidence of CAD was studied.

Methods

Study Design

The present study included 304 subjects (aged >45 years). Seventy-eight patients with angiographically proven CAD have previously been described.[6] Blood samples from these patients were collected from 1993 to 1994 and were analyzed within 1 month after collection. Blood samples from an additional 226 age-matched subjects were collected between January 1998 and May 1999 and were again analyzed within 1 month after collection. One hundred consecutive patients with angiographically proven CAD were recruited at the Cardiology Unit of the University Hospital. Angiograms of CAD patients showed at least 50% stenosis of 1 (n=12 patients), 2 (n=73 patients), or 3 (n=93 patients) coronary arteries. Thirty of 178 CAD patients were scheduled for percutaneous transluminal coronary angioplasty, and 29 patients were scheduled for coronary artery bypass graft. Seventy-six CAD patients had previously suffered from acute coronary syndromes: 15 had unstable angina pectoris, and 61 had experienced a previous acute myocardial infarction. Blood samples were collected >6 months after the last acute event. All patients gave informed consent. One hundred twenty-six age-matched consecutive control subjects without clinical evidence of CAD were recruited at the Endocrinology Unit (A.M., E.M.) between January 1998 and May 1999. The absence of significant atherosclerotic lesions was confirmed by high-resolution

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B-mode ultrasonography[8,9] performed at the Departement van Bloedings-en Vaatziekten of the University Hospital (G.B., R.V.). Laboratory measurements were performed at the Center for Molecular and Vascular Biology and the Center for Experimental Surgery and Anesthesiology (P.H., A.M., and P.V.). Analysis was blinded.

GRAS was calculated on the basis of age, total and HDL cholesterol levels, systolic blood pressure, diabetes mellitus, and smoking. The risk points for different cardiovascular risk factors among men and women were used as previously described.[4] Hypercholesterolemic patients had total cholesterol levels ≥240 mg/dL and/or LDL cholesterol levels ≥130 mg/dL or were treated with cholesterol-lowering statins or fibrates. Dyslipidemic patients with low HDL cholesterol and/or high triglycerides had HDL cholesterol levels <35 mg/dL and/or triglyceride levels in fasting samples >200 mg/dL irrespective of LDL cholesterol levels. Smokers consumed any amount of cigarettes within 1 month before blood sampling, and former smokers had stopped smoking >1 month before blood sampling. Hypertensive patients were untreated patients with systolic pressure of ≥140 mm Hg and/or a diastolic pressure of ≥90 mm Hg and all patients on blood pressure-lowering drugs. Patients with type 2 diabetes included in the present study had been treated with oral antidiabetics and/or insulin for several months or years. Type 1 diabetic patients were not included.

The validity of GRAS and circulating oxidized LDL for CAD has been determined as previously described.[10] The sensitivity was calculated as a/(a+c); the specificity, as d/(b+d); the positive predictive value, as a/(a+b); the negative predictive value, as d/(c+d); the accuracy (percentage), as (a+d)/(a+b+c+d); the likelihood ratio of a positive test, as sensitivity/(1−specificity); and the likelihood ratio of a negative test, as (1−sensitivity)/specificity. Definitions are as follows: a, the number of true positives; b, the number of false positives; c, the number of false negatives; and d, the number of true negatives.

Blood Sampling

Fasting venous blood samples were collected in 0.1 vol of 0.1 mol/L citrate containing 1 mmol/L EDTA, 20 μmol/L vitamin E, 10 μmol/L butylated hydroxytoluene, 20 μmol/L dipyridamole, and 15 mmol/L theophylline. Blood samples were centrifuged at 3000*g* for 15 minutes at room temperature within 1 hour after collection and stored at −30°C until the assays were performed.[5,6,11]

Assays

A monoclonal antibody 4E6–based competition ELISA was used for measuring plasma levels of oxidized LDL.[5,6,11] Monoclonal antibody 4E6 is directed against a conformational epitope in the apoB-100 moiety of LDL that is generated as a consequence of substitution of lysine residues of apoB-100 with aldehydes. The C_{50} values, ie, concentrations that are required to obtain 50% inhibition of antibody binding in the ELISA, are 25 mg/dL for native LDL and 0.025 mg/dL for oxidized LDL, with at least 60 aldehyde-substituted lysines per apoB-100. Total cholesterol, HDL cholesterol, and triglyceride levels were measured by enzymatic methods (Boehringer-Mannheim). LDL cholesterol levels were calculated with the Friedewald formula.[12]

Statistical Analysis

Data are presented as mean±SD. The nonparametric Mann-Whitney test was used when means were compared, and the Fisher exact test was used for analysis of contingency tables. Logistic regression analysis (SPSS version 9.0 for Windows) was performed to assess the relation of CAD to circulating oxidized LDL and GRAS and to independent cardiovascular risk factors. The first multivariate model contained age, sex, hypertension, diabetes type 2, hypercholesterolemia, dyslipidemia, smoking, and body mass index as covariates. The second multivariate model contained levels of circulating oxidized LDL and all other covariates included in the first model. Linear regression analysis was performed to determine the influence of various cardiovascular risk factors on levels of circulating oxidized LDL in subjects without clinical evidence of CAD. A value of $P<0.05$ was considered statistically significant.

TABLE 1. Laboratory Characteristics of Control Subjects and CAD Patients

Characteristics	Control (n=126)	CAD (n=178)	*P*
TC, mg/dL	175±33	183±39	0.062
LDL-C, mg/dL	104±31	114±33	0.0080
HDL-C, mg/dL	47±14	41±13	<0.001
TC/HDL-C	4.03±1.51	4.80±1.54	<0.001
TG, mg/dL	117±48	147±97	0.0015
OxLDL, mg/dL	1.30±0.88	3.11±1.19	<0.001

Data are mean±SD for n subjects.

TC indicates total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TC/HDL-C, total to HDL cholesterol ratio; TG, triglycerides; and OxLDL, oxidized LDL. *P* values of CAD patients vs control subjects were determined by Mann-Whitney test.

Results

Table 1 shows the laboratory characteristics of 304 individuals. Compared with age-matched control subjects, CAD patients had higher LDL cholesterol levels, lower HDL cholesterol levels, higher total cholesterol–to–HDL cholesterol ratios, higher triglyceride levels, and higher circulating oxidized LDL levels. Mean levels of circulating oxidized LDL were similar among men and women. Levels were 1.48±1.05 mg/dL and 1.20±0.74 mg/dL for men and women, respectively, without clinical evidence of CAD compared with 3.15±1.22 mg/dL and 3.03±1.12 mg/dL for men and women, respectively, with CAD. Levels were 3.40±1.21 mg/dL for male CAD patients of group 1 compared with 2.94±1.20 mg/dL for male patients of group 2 ($P=0.044$). Levels were 3.19±0.84 mg/dL for female CAD patients of group 1 compared with 2.72±1.19 mg/dL for female patients of group 2 ($P=0.12$).

Table 2 shows the demographic and clinical characteristics of control subjects and CAD patients. CAD patients were more often male, and they more often had hypercholesterolemia and dyslipidemia and were smokers. GRAS of CAD patients was significantly higher.

TABLE 2. Demographic and Clinical Characteristics of Control Subjects and CAD Patients

Characteristics	Controls (n=126)	CAD (n=178)	*P*
Age, y	59±9.1	60±9.1	0.35
Sex (male/female), n/n	49/77	125/53	<0.001
BMI, kg/m^2	28±8.3	27±4.9	0.19
Hypertension, %	30	24	0.51
Diabetes type 2, %	28	22	0.24
Hypercholesterolemia, %	25	42	<0.001
Treated with statins	2	16	
Treated with fibrates	5	4	
Dyslipidemia, %	17	34	<0.001
Smoker, %	32	54	<0.001
Current	25	39	
Former	7	15	
GRAS	6.13±5.00	8.65±3.41	<0.001

Data indicate percentage of subjects; for age, BMI, body mass index and GRAS, mean±SD is given; and for sex the male/female ratio (n/n) is given.

TABLE 3. Validity of GRAS and of Circulating Oxidized LDL for CAD

	Total		Men		Women	
	Data	Value	Data	Value	Data	Value
GRAS						
Sensitivity, %	35/178	20	30/125	24	9 /78	12
Specificity, %	113/126	90	44 /49	90	69 /77	90
Positive predictive value, %	35 /48	73	30 /35	86	9 /17	53
Negative predictive value, %	113/256	44	44/139	32	69/113	61
Accuracy, %	148/304	49	74/174	43	78/130	1.2
Likelihood ratio of positive test	20 /10	2.0	24 /10	2.4	12 /10	1.2
Likelihood ratio of negative test	80 /90	0.89	76 /90	0.84	88 /90	0.98
Oxidized LDL						
Sensitivity, %	135/178	76	69/126	55	43 /53	81
Specificity, %	113/126	90	44 /49	90	69 /77	90
Positive predictive value, %	135/148	91	70 /75	93	43 /51	84
Negative predictive value, %	113/156	72	44/100	44	69 /79	87
Accuracy, %	248/304	82	114/174	66	111/130	85
Likelihood ratio of positive test	76 /10	7.6	55 /10	5.5	81 /10	8.1
Likelihood ratio of negative test	24 /90	0.27	45 /90	0.50	9 /90	0.1

Sensitivity was calculated as a/(a+c); specificity, as d/(b+d); positive predictive value, as a/(a+b); negative predictive value, as d/(c+d); accuracy, as (a+d)/(a+b+c+d); likelihood ratio of positive test, as sensitivity/one−specificity); and likelihood ratio of negative test, as (one−sensitivity)/specificity, where a is the number of true positives, b is the number of false positives, c is the number of false negatives, and d is the number of true negatives. Cutoff value for GRAS was 12 for the total study population, 10 for men, and 15 for women. Cutoff value for oxidized LDL was 2.30 mg/dL for the total study population, 2.85 mg/dL for men, and 2.13 mg/dL for women. Cutoff values exceeded the 90th percentile of distribution of control subjects.

Levels of oxidized LDL were 2.93±1.17 mg/dL for patients with 1-vessel disease, 2.87±1.41 mg/dL for patients with 2-vessel disease, and 2.84±1.08 mg/dL for patients with 3-vessel disease. Levels of circulating oxidized LDL were 3.19±1.02 mg/dL for patients with previous episodes of unstable angina pectoris, 3.09±1.30 mg/dL for patients with previous acute myocardial infarction, and 3.26±0.93 mg/dL for patients without previous acute coronary syndromes.

At a cutoff value of 12 (value exceeding the 90th percentile of distribution in control subjects), the positive predictive value of GRAS for CAD was 73%, and its negative predictive value was 44%, resulting in an accuracy of 49%. Respective values for men (at a cutoff of 10) were 86%, 32%, and 43%. Respective values for women (at a cutoff of 15) were 53%, 61%, and 60% (Table 3). The likelihood of a positive test was 2.0 for the total study population, 2.4 for men, and 1.2 for women; the likelihood of negative test was 0.89, 0.84, and 0.98, respectively (Table 3).

At a cutoff value of 2.30 mg/dL (value exceeding the 90th percentile of distribution in control subjects), the positive predictive value of circulating oxidized LDL was 91%, and its negative predictive value was 72%, resulting in an accuracy of 82%. Respective values for men (at a cutoff of 2.85 mg/dL) were 93%, 44%, and 66%. Respective values for women (at a cutoff of 2.13 mg/dL) were 84%, 87%, and 85% (Table 3). The likelihood of a positive test was 7.6 for the total study population, 5.5 for men, and 8.1 for women; the likelihood of negative test was 0.27, 0.50, and 0.10, respectively (Table 3). In the present study, total cholesterol level, LDL cholesterol level, and the ratio of total to HDL cholesterol did not discriminate between CAD patients and subjects without cardiovascular disease (data not shown).

Logistic regression analysis revealed that the predictive value of circulating oxidized LDL was additive to that of GRAS (Table 4). The respective odds ratios for GRAS and oxidized LDL were 1.13 ($P=0.0019$) and 5.15 ($P<0.001$) for the total study population, 1.18 ($P=0.027$) and 3.86 ($P<0.001$) for men, and 1.21 ($P=0.001$) and 7.45 ($P<0.001$) for women. Ninety-four percent of the subjects (94% of the men and 100% of the women) with high GRAS and high circulating oxidized LDL had CAD (Table 4).

Table 5 shows the relation of CAD with age, sex, hypertension, diabetes type 2, hypercholesterolemia, dyslipidemia, smoking, body mass index, and circulating oxidized LDL for the entire study population. Inclusion of circulating oxidized LDL in the multivariate model resulted in an increase of R^2 value from 0.22 to 0.67. Overall, 72% of the subjects were predicted correctly by the multivariate model containing established cardiovascular risk factors and oxidized LDL, but only 40% of the subjects were predicted correctly by a model that did not include oxidized LDL.

The relationship between circulating oxidized LDL and cardiovascular risk factors was also studied for subjects without cardiovascular disease. Univariate statistical analysis showed that hypercholesterolemia ($P=0.002$), body mass index ($P=0.002$), dyslipidemia ($P=0.007$), diabetes type 2 ($P=0.010$), and age ($P=0.033$) were correlated with levels of circulating oxidized LDL. In multivariate analysis, hypercholesterolemia ($P=0.003$), body mass index ($P=0.012$), and dyslipidemia ($P=0.024$) were the strongest predictors of circulating oxidized LDL (Table 6). The model explained

TABLE 4. Prediction of CAD With GRAS and Circulating Oxidized LDL

	OR (95% CI)	*P*	Predictive Value, (%)
Total population			
GRAS	1.2 (1.0–1.2)	<0.001	49
Oxidized LDL	5.2 (3.6–7.5)	<0.001	82
GRAS+oxidized LDL			
GRAS	1.1 (1.0–1.2)	0.0019	94
oxidized LDL	5.2 (3.6–7.5)	<0.001	
Men			
GRAS	1.2 (1.1–1.4)	<0.001	43
Oxidized LDL	4.1 (2.6–6.5)	<0.001	61
GRAS+oxidized LDL			
GRAS	1.2 (1.0–1.4)	0.027	94
oxidized LDL	3.9 (2.4–6.1)	<0.001	
Women			
GRAS	1.2 (1.1–1.3)	<0.001	60
Oxidized LDL	6.6 (3.6–12)	<0.001	73
GRAS+oxidized LDL			
GRAS	1.2 (1.1–1.4)	0.0010	100
oxidized LDL	7.5 (3.7–14)	<0.001	

OR indicates odds ratio. Classification cutoff was 0.9.

30% of variation of oxidized LDL compared with 70% for a model that also contained angiographically proven CAD.

Discussion

We determined the usefulness of circulating oxidized LDL levels for identifying CAD patients. At a cutoff value exceeding the 90th percentile of distribution for control subjects, the likelihood of a positive test for circulating oxidized LDL among patients with angiographically proven CAD was 7.6 (5.5 for men and 8.1 for women). This value was 3.8 times (2.3 for men and 6.7 for women) higher than that of GRAS. The predictive value of oxidized LDL was additive to that of GRAS, and 94% of the subjects with high circulating oxidized LDL levels and high GRAS had CAD. These data indicate that the circulating oxidized LDL level is a useful marker for identifying patients with CAD.

TABLE 5. Logistic Regression Analysis of Relationship Between CAD and Potential Cardiovascular Risk Factors

Covariate	χ^2	*P*	OR (95% CI)
Multivariate model 1			
Male sex	15	<0.001	3.1 (1.8–5.6)
Dyslipidemia	8.2	0.0042	2.6 (1.4–5.0)
Age	6.1	0.013	1.1 (1.0–1.1)
Hypercholesterolemia	4.3	0.037	1.9 (1.0–3.4)
Multivariate model 2			
Oxidized LDL	59	<0.001	7.0 (4.3–11)
Dyslipidemia	6.0	0.014	3.0 (1.3–7.4)
Male sex	6.2	0.013	3.1 (1.3–7.7)
Age	4.3	0.038	1.1 (1.0–1.1)

The multivariate model 1 contained age, sex, hypertension, diabetes type 2, hypercholesterolemia, dyslipidemia, smoking, and BMI as covariates. The R^2 value of this model was 0.22. Overall, 40% of the patients were predicted correctly at a classification cutoff of 0.9. The multivariate model 2 contained levels of circulating oxidized LDL and all other covariants included in the first model. The R^2 value of this model was 0.67. Overall, 72% of patients were predicted correctly at a classification cutoff of 0.9.

TABLE 6. Linear Regression Analysis of Relationship Between Oxidized LDL and Cardiovascular Risk Factors in Subjects Without Clinical Evidence of CAD

	Univariate Analysis		Multivariate Analysis	
	R	*P*	F	*P*
Hypercholesterolemia	0.22	0.002	8.8	0.003
BMI	0.21	0.002	6.5	0.012
Dyslipidemia	0.18	0.007	5.2	0.024
Diabetes type 2	0.17	0.010		
Age	0.13	0.033		

Stepwise multivariate regression analysis was performed. Hypercholesterolemia was correlated with diabetes type 2 (r=0.25, P=0.001), dyslipidemia (r=0.20, P=0.001), age (r=0.19, P=0.001), and smoking (r=0.11, P=0.027). BMI was correlated with dyslipidemia (r=0.26, P<0.001), age (r=0.19, P=0.001), female sex (r=0.19, P=0.002), diabetes type 2 (r=0.13, P=0.021), and hypertension (r=0.12, P=0.029). The model explained 30% of the variation of oxidized LDL.

The relationship between circulating oxidized LDL levels and cardiovascular risk factors was determined for subjects without clinical evidence of cardiovascular disease. Circulating oxidized LDL was correlated with hypercholesterolemia, body mass index, diabetes type 2, and age. In stepwise multivariate analysis, hypercholesterolemia and obesity were the strongest predictors of the circulating oxidized LDL level. Recently, the American Heart Association recognized obesity as an independent cardiovascular risk factor.[13] As expected, obesity was associated with dyslipidemia, age, diabetes type 2, and hypertension, which are frequently observed in association with obesity.[14,15] It remains to be investigated whether the increase of circulating oxidized LDL is due to the occurrence of small dense LDL, which is associated with obesity and which is an integral feature of insulin resistance, a proposed risk factor for CAD.[16–19] Although the control subjects had no angiograms, the absence of significant atherosclerotic lesions in the carotid arteries was confirmed by ultrasonography. Recently, we have demonstrated that obese subjects without clinical evidence of CAD but with increased intima/media thickness of carotid arteries had increased circulating oxidized LDL that was correlated with intima/media thickness, suggesting that an increased oxidized LDL level is a marker of early atherosclerosis (authors' unpublished data).

Thus, the present study shows a strong association between oxidized LDL and CAD and a significant correlation between oxidized LDL and most of the Framingham risk factors. Therefore, the study of the relationship between oxidized LDL and the development of cardiovascular disease is warranted. Recently, we have demonstrated that baseline levels of circulating oxidized LDL predict the development of transplant-associated CAD.[20] In animal models of coronary artery atherosclerosis,[21,22] the accumulation of oxidized LDL in the intima of the coronary arteries preceded the development of coronary plaques.

The limitation of the present study is that coronary stenosis was assessed by angiography and not by more sensitive methods, such as intravascular ultrasound. Therefore, we did not attempt to study the relationship between plaque load and levels of circulating oxidized LDL. The relationship between circulating oxidized LDL and other potential risk factors, such as Lp(a) and homocysteine, has not yet been studied. However, to date, data demonstrating the additive value of Lp(a) and homocysteine to lipid measurement for cardiovascular risk prediction are inconsistent. Recently, measurement of high sensitivity C-reactive protein in addition to lipid measurement has been shown to cause significant improvement in clinical prediction models based on lipids alone in men and women.[23,24] However, high sensitivity measurements of C-reactive protein were not possible in the present study.

In aggregate, the present study shows that circulating oxidized LDL is a sensitive marker of CAD that is correlated with most of the Framingham risk factors. Inclusion of circulating oxidized LDL in prospective studies of risk factors of CAD is warranted. Our recent finding that circulating oxidized LDL is a prognostic marker of transplant-associated CAD further suggests that oxidized LDL may indeed play a causative role in coronary atherosclerosis.

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