Improved Identification of Patients With Coronary Artery Disease by the Use of New Lipid and Lipoprotein Biomarkers

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Increasing attention is being directed toward new lipid and lipoprotein biomarkers as risk factors for coronary artery disease, although limited information is available on the diagnostic accuracy of these new biomarkers for the identification of patients with coronary artery disease. In the present study, levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, lipoprotein-associated phospholipase A2 (Lp-PLA2), and oxidized LDL/HDL cholesterol were determined in 431 apparently healthy men and women without clinical evidence of coronary artery disease who were matched for age and gender with 490 men and women with coronary artery disease who participated in the Second Fragmin and Fast Revascularization During Instability in Coronary Artery Disease (FRISC-II) trial. Diagnostic accuracy was determined by receiver-operating characteristic curve analysis by measuring the area under the curve. The diagnostic accuracies of each lipid or lipoprotein biomarker (in descending order of area under the curve) were 0.867 for oxidized LDL/HDL cholesterol (95% confidence interval [CI] 0.844 to 0.890), 0.826 for oxidized LDL (95% CI 0.800 to 0.852), 0.775 for 1/HDL cholesterol (95% CI 0.745 to 0.805), 0.764 for total/HDL cholesterol (95% CI 0.733 to 0.795), 0.631 for triglycerides (95% CI 0.594 to 0.667), 0.597 for Lp-PLA2 (95% CI 0.558 to 0.615), 0.577 for LDL cholesterol (95% CI 0.539 to 0.615), and 0.520 for total cholesterol (95% CI 0.482 to 0.537). In conclusion, these findings indicate that the ratio of oxidized LDL to HDL cholesterol was a more potent biomarker for discriminating between subjects with and without coronary artery disease than traditionally measured lipids and lipoproteins and Lp-PLA2.   © 2006 Elsevier Inc. All rights reserved. (Am J Cardiol 2006;97:640–645)

Recently, oxidized low-density lipoprotein (LDL), an atherogenic form of LDL cholesterol, and lipoprotein-associated phospholipase A2 (Lp-PLA2), an enzyme linked to LDL with proinflammatory properties, have been identified as possible biomarkers for coronary artery disease. These 2 biomarkers appear to be high in patients with coronary artery disease, and it has been suggested that their levels may predict the risk of future cardiac events.[1–8] In this study, we compared the diagnostic accuracies of these new biomarkers with those of traditionally measured lipids and lipoproteins to determine their ability to discriminate between patients with and without coronary artery disease. Diagnostic accuracy was determined using receiver-operating characteristic curve analysis, which is widely accepted as the standard statistical method for comparing accuracies of medical diagnostic tests.[9,10]

Methods

Study populations: The patient population consisted of 490 patients with unstable coronary artery disease (i.e., unstable angina or non–ST-elevation myocardial infarction) who were included in the Second Fragmin and Fast Revascularization During Instability in Coronary Artery Disease (FRISC-II) trial. These patients came from 6 trial centers in Sweden that were willing to participate in this substudy. The design, objectives, and main results of the FRISC-II trial have been described previously.[11]

The control population consisted of 431 subjects who were included in the Sweden Women and Men and Ischemic Heart Disease (SWISCH) study.[12] The SWISCH study was a case-control study of risk factors for coronary artery disease in elderly men and women. Subjects were recruited from a pool of subjects who were randomly matched by age and gender to patients in the FRISC-II trial using the population registry. Subjects were initially sent a survey by mail. Subjects who reported freedom from cardiovascular disease, other chronic disease, and use of cardiovascular medication, including lipid-lowering agents, were then asked to participate

in an outpatient visit. At the outpatient visit, subjects underwent a clinical examination, including heart auscultation, measurement of blood pressure, height and weight, electrocardiography, and blood sampling. Subjects who were included in the final sample had normal electrocardiograms and routine laboratory blood chemistry, including hemoglobin, white blood cell count, platelet count, creatinine and blood glucose levels and were not acutely ill.

All patients and control subjects gave written informed consent, and the study was approved by the ethics committees of all participating hospitals.

Laboratory analyses: In patients, blood samples that were used to analyze oxidized LDL and Lp-PLA2 were obtained at inclusion, which was a median of 33 hours after the final ischemic episode. Blood samples that were used to measure total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were obtained on the morning after study inclusion. In control subjects, blood samples were obtained at an outpatient visit. Venous blood was drawn from a cubital vein with the subject seated and then collected in tubes that contained ethylene-diaminetetra-acetic acid or citrate. After centrifugation at 2,000*g* for 20 minutes at room temperature within 30 minutes of collection, plasma was frozen in aliquots and stored at −70°C until analysis. Routine blood chemistry and lipids (total cholesterol, HDL cholesterol, and triglycerides) were analyzed using fresh blood samples according to established enzymatic methods at the local laboratories. Levels of LDL cholesterol were calculated with Friedewald's formula.[13] Levels of oxidized LDL and Lp-PLA2 were analyzed at the core laboratory for the FRISC-II study in Uppsala, Sweden. A sandwich enzyme-linked immunosorbent assay was used to measure plasma levels of oxidized LDL (Mercodia AB, Uppsala, Sweden). Assay precision was calculated from 12 samples that were assayed in 3 replicates on 2 different occasions. The within-run and between-run coefficients of variation were 8.9% and 9.2%, respectively. Plasma levels of Lp-PLA2 were determined with a commercial Lp-PLA2 kit (diaDexus, Inc., San Francisco, California). The lower detection limit of this assay is 2 ng/ml, and the between-run coefficient of variation is 9.6%.

Statistical analyses: Continuous variables are presented as mean ± SD. Data were analyzed as independent random samples because the numbers of controls and patients differed due to the response rate of the mail survey and the exclusion criteria applied at the outpatient visit. Significance of observed mean differences were tested with Student's *t* test and were calculated with SAS 8.02 for Windows (SAS Institute, Cary, North Carolina). Receiver-operating characteristic curve analyses were used to evaluate the diagnostic accuracies of the various markers. These were generated with Analyze-it 1.67 (Analyze-It, Leeds, United Kingdom), which calculated the area under the curve, 95% confidence intervals (CIs) for the area under the curve, sensitivity, and specificity. The receiver-operating characteristic curve was
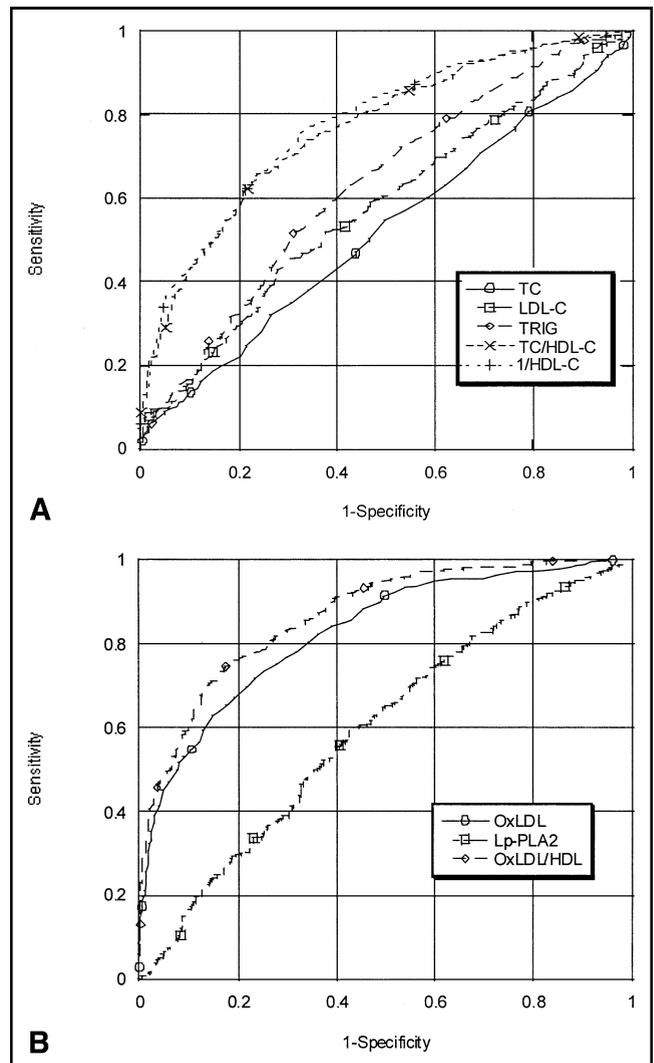

Figure 1. Receiver-operating characteristic curves for *(A)* traditional lipids and lipoproteins and *(B)* emerging lipid and lipoprotein biomarkers. HDL-C = HDL cholesterol; LDL-C = LDL cholesterol; OxLDL = oxidized LDL; TC = total cholesterol; TRIG = triglyceride.

constructed by plotting sensitivity on the y axis and the proportion of false positives (1 − specificity) on the x axis. The area under the receiver-operating characteristic curve varies from 1.0, which corresponds to perfect discrimination (upper left corner), to 0.5, which indicates no discrimination. Data from Analyze-it were used to generate the overlaid receiver-operating characteristic curves shown in Figure 1 with KaleidaGraph 3.52. In addition, data were manipulated in Microsoft Excel 2002 (Microsoft, Redmond, Washington) to calculate positive and negative predictive values, odds ratios, and 95% CIs for the odds ratio for each factor.

Results

Characteristics of the study populations: Baseline characteristics of healthy controls and patients with coronary

Table 1
Baseline characteristics of healthy controls and patients with coronary artery disease

Variable	Controls (n = 431)	Patients (n = 490)
Women	137 (32%)	141 (29%)
Age (yrs)	64.7 ± 8.9	64.7 ± 8.9
Body mass index (kg/m^2)	25.4 ± 3.3	27.1 ± 3.7§
Current smoker	60 (14%)	121 (25%)§
Diabetes mellitus	0	58 (12%)
Hyperlipidemia*	0	48 (10%)
Hypertension*	0	161 (33%)

Values are mean ± SD or numbers of subjects percentages.

* Defined as pharmacologic treatment for this condition.

† p <0.05; ‡ p <0.01; § p <0.001 (controls vs patients).

artery disease are presented in Table 1. Mean plasma levels for the various biomarkers are listed in Table 2. Quintiles of the control population were derived for each biomarker, and the distribution of patients with coronary artery disease across these quintiles was examined. Percentages of patients in the lowest (quintile 1) and highest (quintile 5) quintiles of the control group are presented in Figure 2. The greatest contrast in the percentages of patients in quintiles 1 and 5 was noted for oxidized LDL/HDL cholesterol.

Variables associated with coronary artery disease: Table 3 presents univariate odds ratios for the risk of having coronary artery disease for a positive versus negative test result (i.e., above the cut-off value presented in Table 4). All lipid and lipoprotein biomarkers except for total cholesterol were associated with a statistically significant increase in risk. Oxidized LDL/HDL cholesterol was the biomarker that was associated with the most significant increase in risk.

Receiver-operating characteristic analyses: Table 4 presents the cutoffs, sensitivities, specificities, negative and positive predictive values, and areas under the curve for the various markers. Figure 1 shows the receiver-operating characteristic curves for the traditional lipids and lipoproteins. Figure 1 also presents the receiver-operating characteristic curves for the emerging lipid and lipoprotein biomarkers. The biomarker with the highest diagnostic accuracy in distinguishing healthy controls from patients with coronary artery disease was oxidized LDL/HDL cholesterol. At a cut-off value of 53, sensitivity was 76% and specificity was 82%, with a corresponding area under the curve of 0.867 (95% CI 0.844 to 0.890). Other biomarkers with good diagnostic accuracy (area under the curve >0.7) were oxidized LDL, total cholesterol/HDL cholesterol, and HDL cholesterol. Oxidized LDL/HDL cholesterol was a significantly better discriminator between patients with coronary artery disease and control subjects than were all traditional lipid and lipoprotein biomarkers, based on the lack of overlap of the CIs as presented in Tables 3 and 4. Levels of total cholesterol, LDL cholesterol, triglycerides,

or Lp-PLA2 were not particularly helpful in discriminating patients with coronary artery disease from matched healthy control subjects (area under the curve <0.7).[14]

Discussion

In this study, we compared the diagnostic accuracies of 8 different lipid or lipoprotein biomarkers that have been suggested to identify patients with an increased risk and occurrence of coronary artery disease. The study focused on diagnostic information that was obtained from a comparison of levels of lipid and lipoprotein biomarkers between age- and gender-matched, apparently healthy, patients without clinical evidence of coronary artery disease and patients with established coronary artery disease. Diagnostic accuracy was determined by measuring the area under the curve of the receiver-operating characteristic curve, which is the most common method for quantifying and comparing the accuracies of different diagnostic tests.

We have demonstrated, for the first time, by receiver-operating characteristic curve analysis that oxidized LDL, especially when used in combination with HDL cholesterol, is a better biomarker than standard lipid measurements for discriminating between patients with coronary artery disease and healthy subjects. In addition, we have shown that levels of another new lipoprotein biomarker, Lp-PLA2, provided no information to discriminate patients with coronary artery disease from healthy subjects. Further, levels of total cholesterol, LDL cholesterol, and triglycerides discriminated poorly between patients with coronary artery disease and healthy subjects.

Currently, there are 3 other published studies[1–3] in which oxidized LDL and traditional lipid or lipoprotein biomarkers were measured in patients with coronary artery disease and compared with levels in control subjects without coronary artery disease. These studies differed from our study in a number of ways. First, in these studies, oxidized LDL was measured using a different assay procedure or a different monoclonal antibody than that used in our study. We used a sandwich-type enzyme-linked immunosorbent assay and the monoclonal antibody 4E6, which is directed against cross linkages between amino-lysine groups of apolipoprotein B, the protein moiety of LDL, and reactive aldehydes that result from oxidation of long-chain fatty acids. In contrast, Holvoet et al[1] used the same monoclonal antibody (4E6) but also a competitive enzyme-linked immunosorbent assay. There seems to be a small but significant difference between results obtained with a sandwich-type enzyme-linked immunosorbent assay and those obtained with a competitive enzyme-linked immunosorbent assay; the correlation coefficient between the 2 methods is 0.75, as determined by the manufacturer of the 2 kits. Toshima et al[2] and Suzuki et al[3] used sandwich-type enzyme-linked immunosorbent assays; however, they used the monoclonal antibody FOH1a/DLH3, which is directed against oxidized

Table 2
Levels of lipid and lipoprotein biomarkers in the study populations

Lipid or Lipoprotein	Control Group (mean ± SD)	CAD Group (mean ± SD)	Mean Difference (%)	p Value
Total cholesterol				
mg/dl	221 ± 38.7	225 ± 42.5	1.75	0.179
mmol/L	5.71 ± 1.00	5.81 ± 1.10		
LDL*				
mg/dl	134 ± 34.8	145 ± 41.0	8.07	<0.0001
mmol/L	3.47 ± 0.90	3.75 ± 1.06		
Lp-PLA2 (ng/ml)	278 ± 97	310 ± 191	11.51	0.0025
Triglycerides				
mg/dl	154 ± 80.5	191 ± 100	24.14	<0.0001
mmol/L	1.74 ± 0.91	2.16 ± 1.13		
1/HDL				
dl/mg	0.019 ± 0.005	0.025 ± 0.009	32.88	<0.0001
L/mmol	0.73 ± 0.19	0.97 ± 0.33		
Total cholesterol/HDL (ratio)	4.12 ± 1.16	5.57 ± 2.01	35.19	<0.0001
Oxidized LDL (U/L)	53.82 ± 14.34	76.21 ± 19.48	41.60	<0.0001
Oxidized LDL/HDL (ratio)	39.70 ± 16.33	74.07 ± 31.12	86.57	<0.0001

CAD = coronary artery disease.
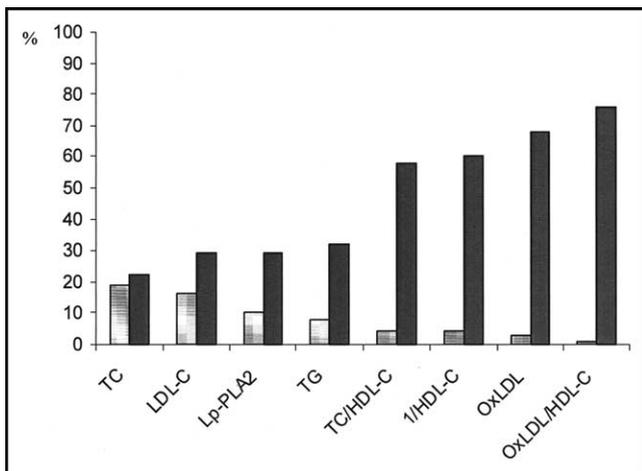
* Available in 470 patients and 417 controls.

Figure 2. Percentages of patients in the lowest quintile *(gray bars)* and highest quintile *(black bars)* of the control group for each lipid or lipoprotein biomarker (total cholesterol: quintile 5 >6.6 mmol/L, quintile 1 <4.8 mmol/L; LDL cholesterol: quintile 5 >4.18 mmol/L, quintile 1 <2.74 mmol/L; triglycerides [TGs]: quintile 5 >2.30 mmol/L, quintile 1 <1.06 mmol/L; Lp-PLA2: quintile 5 >340.00 pg/ml, quintile 1 <206.35 pg/ml; 1/HDL cholesterol: quintile 5 >0.91; quintile 1 >0.56; total/HDL cholesterol: quintile 5 >5.00, quintile 1 >3.13; oxidized LDL: quintile 5 >66, quintile 1 <42; oxidized LDL/HDL cholesterol: quintile 5 >52.5, quintile 1 <25.63). Abbreviations as in Figure 1.

Table 3
Unadjusted odds ratios and 95% confidence intervals for coronary artery disease for various lipid and lipoprotein biomarkers

	OR (95% CI)
Total cholesterol	1.20 (0.93–1.56)
LDL	1.90 (1.44–2.51)
Lp-PLA2	2.02 (1.54–2.66)
Triglycerides	2.34 (1.79–3.05)
Total cholesterol/HDL	6.12 (4.56–8.20)
1/HDL	6.61 (4.93–8.86)
Oxidized LDL	8.26 (6.15–11.11)
Oxidized LDL/HDL	13.92 (10.07–19.23)

OR = odds ratio.

phosphatidylcholines. Second, our patient and control populations differed in demographic and clinical characteristics from the study populations in these other studies. Third, none of the other studies evaluated the oxidized LDL/HDL cholesterol ratio, which we found to be a better indicator of coronary artery disease than any of the other lipid or lipoprotein biomarkers. Fourth, none of the other studies compared the diagnostic information on oxidized LDL level with the levels of another new biomarker, such as Lp-PLA2.

The greater diagnostic accuracy of oxidized LDL versus native LDL cholesterol in identifying patients with coronary artery disease could be related to oxidized LDL being more directly involved than native LDL cholesterol in the atherosclerotic disease process. Oxidative modification of LDL cholesterol greatly increases the conversion of monocytes or macrophages in the arterial wall into cholesterol-laden foam cells, which are an essential component of atherosclerotic plaque.[15,16] Further, oxidized LDL has other biologic properties that native LDL cholesterol does not, e.g., oxidized LDL promotes chemotaxis of monocytes and lymphocytes and modulates growth factor and cytokine production from endothelial cells, smooth muscle cells, and macrophages.[17]

The diagnostic accuracy of oxidized LDL was further improved with the combined use of HDL cholesterol. This finding is in line with other studies in which lipid and apolipoprotein ratios that included an atherogenic and an antiatherogenic lipid component have been strongly associated with coronary artery disease.[18] Although oxidized LDL is involved in the deposition of cholesterol in the arterial wall, HDL cholesterol is involved in its removal.[19] In addition, HDL cholesterol may prevent oxidation of LDL

Table 4
Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under the curve (AUC) for each lipid and lipoprotein biomarker

	Optimal Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (95% CI)
Total cholesterol	220 mg/dl	55	50	55	49	0.520 (0.482–0.557)
LDL	148 mg/dl	46	69	62	54	0.577 (0.539–0.615)
Lp-PLA2	268 pg/ml	60	58	64	53	0.597 (0.558–0.615)
Triglycerides	143 mg/dl	63	58	63	58	0.631 (0.594–0.667)
Total cholesterol/HDL	4.8	66	76	75	67	0.764 (0.733–0.795)
1/HDL	0.02 dl/mg	76	68	72	72	0.775 (0.745–0.805)
Oxidized LDL	63 U/L	74	75	77	71	0.826 (0.800–0.852)
Oxidized LDL/HDL	53	76	82	82	76	0.867 (0.844–0.890)

cholesterol and may also interfere with the pathophysiologic action of oxidized LDL.[20]

We have found that total cholesterol and LDL cholesterol have poor diagnostic accuracy in identifying patients with coronary artery disease. Other published studies agree with our findings.[1–3,21] Total cholesterol does not accurately discriminate patients with coronary artery disease from healthy subjects because it is the sum of cholesterol carried by atherogenic (LDL, very LDL, and intermediate-density lipoprotein) and antiatherogenic (HDL) lipoproteins. LDL cholesterol is often calculated with Friedewald's equation (LDL cholesterol = total cholesterol − HDL cholesterol − [triglycerides/5]).[13] In addition, calculation of LDL cholesterol by Friedewald's equation and direct determination of LDL cholesterol by immunoassay fail to account for the heterogeneity of LDL cholesterol, which affects the physical properties of LDL cholesterol particles.[22]

There are some limitations to this study that need to be considered. The control population consisted of apparently healthy patients, and the patient population consisted of patients with acute coronary syndromes. Although this may not be an optimal matching of cases and controls to evaluate the importance of different biomarkers in identifying patients with coronary artery disease, there are studies that suggest that levels of these markers are not significantly affected by an acute coronary event. Concerning traditional lipids, there is some evidence to suggest that levels of total cholesterol, LDL cholesterol, and HDL cholesterol decrease and levels of triglycerides increase in the postinfarction period, with maximal changes occurring 4 to 7 days after infarction.[23] We obtained blood samples within 2 days of an ischemic event, and our results imply that levels of lipids and lipoproteins at this time provide a relatively accurate assessment. Therefore, we use these values in clinical practice. Little is known about the kinetics of oxidized LDL during an acute coronary event. However, Holvoet et al[24] used an antibody similar to ours and found that oxidized LDL levels were increased to similar levels in patients with stable angina and acute myocardial infarction, suggesting that this epitope may be a marker of a long-term process. These findings are in contrast to those of another study that used an antibody directed at a different oxidation epitope. Ehara et al[25] found that levels of oxidized LDL appeared to

be related to the severity of an acute coronary syndrome. Currently, it is unclear which epitopes of oxidized LDL are relevant to the different stages of the atherosclerotic disease process.[26] Brislakis et al[7] found no differences in levels of Lp-PLA2 between patients with stable coronary artery disease and those with unstable disease.

An additional limitation of this study is that we did not adjust for major clinical risk factors. This was due to the design of the 2 studies and the definitions that were used to classify risk factors. However, we believe our study findings are valid despite this lack of correction because whatever bias may be introduced by the patient group having more high-risk factors applies to all the biomarkers investigated. Our study serves mostly to emphasize the need for a definitive prospective study on these markers.

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