Relationship of IgG and IgM autoantibodies to oxidized low density lipoprotein with coronary artery disease and cardiovascular events

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Abstract The relationship between autoantibodies to oxidized low density lipoprotein (OxLDL) and coronary artery disease (CAD) remains controversial. IgM and IgG OxLDL autoantibodies to malondialdehyde (MDA)-modified LDL, copper oxidized low density lipoprotein (CuOxLDL), and oxidized cholesterol linoleate (OxCL), as well as apolipoprotein B-100 immune complexes (apoB-ICs), were measured in 504 patients undergoing clinically indicated coronary angiography. Patients were followed for cardiovascular events for a median of 4 years. In univariate analysis, IgM OxLDL autoantibodies and IgM apoB-ICs were inversely associated with the presence of angiographically determined CAD, whereas IgG OxLDL autoantibodies and IgG apoB-ICs were positively associated. In logistic regression analysis, compared with the first quartile, patients in the fourth quartile of IgM OxLDL autoantibodies and apoB-ICs showed a lower probability of angiographically determined CAD (>50% diameter stenosis). Odds ratios and (95% confidence intervals) were as follows: MDA-LDL, 0.51 (0.32–0.82; $P = 0.005$); CuOxLDL, 0.63 (0.39–1.01; $P = 0.05$); OxCL, 0.63 (0.39–1.01; $P = 0.05$); and apoB-IC, 0.55 (0.34–0.88; $P = 0.013$). These relationships were accentuated in the setting of hypercholesterolemia, with the highest IgM levels showing the lowest risk of CAD for the same level of hypercholesterolemia. Multivariable analysis revealed that neither IgM or IgG OxLDL autoantibodies nor apoB-ICs were independently associated with angiographically determined CAD or cardiovascular events. In conclusion, IgG and IgM OxLDL biomarkers have divergent associations with CAD in univariate analysis but are not independent predictors of CAD or clinical events.—Tsimikas, S., E. S. Brilakis, R. J. Lennon, E. R. Miller, J. L. Witztum, J. P. McConnell, K. S. Kornman, and P. B. Berger. **Relationship of IgG and IgM autoantibodies to oxidized low density lipoprotein with coronary artery disease and cardiovascular events. *J. Lipid Res.* 2007.** 48: **425–433.**

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Oxidative modification of LDL is postulated to be one of the earliest events in the initiation of atherogenesis (1, 2). Oxidized low density lipoprotein (OxLDL) is present in aortas of human fetuses of hypercholesterolemic mothers (3) even before macrophage foam cell formation, in progressing atherosclerotic lesions of animal models (4), and within human vulnerable plaques (5, 6). Conceptually, OxLDL is not a single defined chemical entity but represents a variety of modifications of both the lipid and protein components of LDL after the initiation of lipid peroxidation. The resulting oxidized lipid and oxidized lipid-protein adducts are not only proinflammatory (7) but also are recognized as foreign by the immune system and therefore are highly immunogenic (8).

Although autoantibodies to OxLDL epitopes correlate well with atherosclerosis in animal models (9–11), an ongoing controversy exists regarding whether OxLDL autoantibodies are markers of cardiovascular disease and/or have a causative role in either the progression of or protection against the development of atherosclerosis in humans (12). Much of this controversy is fueled by many factors, including measurement of different OxLDL epitopes, underpowered studies and incomplete assessment of different OxLDL autoantibodies, differences among patient cohorts, comparison of different vascular areas, and lack of standardization of antigens and assays. In this large cross-sectional study of 504 patients, we evaluated a comprehensive panel of both IgG and IgM OxLDL autoantibodies to a wide array of OxLDL epitopes, including

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malondialdehyde (MDA)-LDL, copper oxidized low density lipoprotein (Cu-OxLDL), and oxidized cholesterol linoleate (OxCL), as well as apolipoprotein B-100 immune complexes (apoB-ICs), and their relationship to angiographically determined coronary artery disease (CAD).

METHODS

Study design

The patient cohort included 504 patients (>97% Caucasian), age 26–75 years, undergoing clinically indicated coronary angiography at the Mayo Clinic from June 1998 to January 1999 and has been described previously (13). The initial study was designed to assess genetic polymorphisms in cardiovascular risk assessment. Therefore, patients with well-established risk factors or risk equivalents, such as diabetes mellitus and smoking history of >50 pack years, as well as prior revascularization procedures, were excluded. This study was approved by the Mayo Clinic Institutional Review Board, and all subjects provided written informed consent. Aliquots of blood in EDTA-containing tubes were collected before coronary angiography and frozen at −70°C until analyses were performed.

Four hundred sixty-six patients (92.5%) were contacted by a follow-up questionnaire or by telephone in September 2002 [median follow-up of 4.0 years (interquartile range, 3.9–4.2 years)]. The remaining 38 patients either refused to participate in the follow-up (n = 18) or could not be contacted (n = 20). The medical records of the patients who had an event were obtained and reviewed to ascertain the type of event or the cause of death. The follow-up events of these patients were described previously (14) and consisted of 20 deaths (6 cardiac), 14 myocardial infarctions, 26 coronary revascularizations (15 percutaneous intervention only, 9 coronary artery bypass surgery only, and 2 with both), and 10 strokes.

Angiographic analysis

Coronary angiograms were analyzed as described previously (13) and divided into those revealing normal coronary arteries [smooth arteries with either no stenosis or with diameter stenosis (DS) ⩽ 10%], mild disease (DS 10–50% in one or more coronary arteries), one-vessel (⩾50% DS in a single coronary artery or its major branches), two-vessel, and three-vessel disease.

Laboratory analyses

Total cholesterol, HDL cholesterol (HDL-C), and triglycerides were determined on a COBAS MIRA system. LDL cholesterol (LDL-C) was estimated from the Friedewald formula. High-sensitivity C-reactive protein (hsCRP) was measured as described previously (15). Hypercholesterolemia was defined as total cholesterol ⩾ 250 mg/dl or LDL ⩾ 150 mg/dl, or ongoing treatment with lipid-lowering agents in patients for whom pretreatment lipid values could not be determined. Fibrinogen was measured by an immunoturbidimetric method using reagents from Kamiya Biomedical Co. on a Roche COBAS MIRA chemistry analyzer (absorbance, 700 nm). Lipoprotein-associated lipoprotein lipase A_2 mass was measured with a commercial kit (Diadexus, Inc., South San Francisco, CA). Total plasma homocysteine was measured after reduction of the disulfide bonds by high-pressure liquid chromatography.

Determination of OxLDL autoantibody and apoB-IC levels

Chemiluminescence ELISAs were used to measure IgG and IgM autoantibodies to MDA-LDL, Cu-OxLDL, OxCL, and apoB-IC as described previously (16, 17). OxLDL-E06, measuring the oxidized phospholipid content on apoB particles, was measured as described previously (13, 17).

Statistical methods

Continuous variables with symmetric distributions are summarized as means ± SD and are compared by Student's *t*-test. Those with skewed distributions are summarized as medians and interquartile ranges and compared with the Mann-Whitney rank sum test. Discrete covariates are summarized as frequencies and group percentages and are compared using Pearson's Chi-square test. Spearman's correlation coefficient was used to measure the linear association between the rank values of OxLDL autoantibodies and apoB-IC and clinical and laboratory variables.

The association between OxLDL autoantibodies and CAD was assessed using logistic regression analysis. The laboratory values were collapsed into quartiles and assigned a value from 1 to 4. These values were then used in the logistic regression models. There was no evidence of a nonlinear trend over quartiles in any model. Models with OxLDL autoantibodies and hypercholesterolemia assume additivity between the two variables; there was no evidence of an interaction in any such model. Multiple logistic regression was used to estimate the partial associations between OxLDL autoantibodies and CAD adjusting for age, sex, smoking history, hypertension, LDL-C, HDL-C, log(triglycerides), and log(hsCRP).

RESULTS

Baseline patient characteristics

The baseline clinical characteristics, indications for coronary angiography, and lipid parameters of the study cohort have been described previously (13). For the purposes of this analysis, patients were dichotomized as those with <50% DS or >50% DS, and baseline characteristics are shown in **Table 1**. There were 233 patients with nonobstructive CAD (<50% DS) and 271 with obstructive CAD (>50% DS). Of these, 122 patients had angiographically normal coronary arteries, 111 had mild CAD (10–50% DS), and 85, 80, and 106 had one-, two-, and three-vessel CAD, respectively (⩾50% DS). As expected, patients with obstructive CAD were more likely to be older and male and had a higher frequency of hypertension and dyslipidemia (Table 1). Indications for angiography reflected typical clinical indications as described previously (13).

Association of OxLDL autoantibody and apoB-IC levels with angiographically determined CAD

OxLDL autoantibodies and apoB-ICs were evaluated according to whether patients had >50% DS versus <50% DS and also as no CAD (angiographically normal arteries) versus any CAD (**Table 2**). In both analyses, IgM autoantibody levels to MDA-LDL, Cu-OxLDL, and OxCL as well as IgM apoB-IC were significantly lower in patients with obstructive versus nonobstructive CAD as well as any CAD versus no CAD (Table 2). In contrast, IgG autoantibody levels to Cu-OxLDL were higher in patients with obstructive versus nonobstructive CAD and any CAD versus no CAD (Table 2). The other IgG measures were not statistically significantly different among groups.

TABLE 1. Baseline clinical and laboratory characteristics of patients with no or mild angiographically determined CAD and those with obstructive CAD (>50% DS)

Variable	No/Mild CAD (n = 233)	CAD (n = 271)	*P*
Age, years	57.6 ± 11.3	62.2 ± 10.2	<0.001
Female, n (%)	131 (56%)	62 (23%)	<0.001
Hypertension, n (%)	89 (38%)	143 (53%)	0.001
Current smoker, n (%)	17 (7%)	23 (8%)	0.62
Prior myocardial infarction, n (%)	9 (4%)	68 (25%)	<0.001
Congestive heart failure, n (%)	44 (19%)	15 (6%)	<0.001
Family CAD history, n (%)	51 (22%)	77 (28%)	0.09
Hypercholesterolemia, n (%)	96 (41%)	190 (70%)	<0.001
Treated with statin, n (%)	35 (15%)	107 (39%)	<0.001
Body mass index	29.0 ± 6.3	29.6 ± 4.8	0.24
Creatinine, median (Q1, Q3)	1.1 (1.0, 1.2)	1.2 (1.0, 1.3)	<0.001
Total cholesterol, mg/dl	203.8 ± 41.9	210.5 ± 47.1	0.10
Triglycerides, median (Q1, Q3)	150.0 (109.0, 202.0)	157.0 (114.0, 213.0)	0.13
HDL cholesterol, mg/dl	52.4 ± 16.3	44.0 ± 11.5	<0.001
LDL cholesterol, mg/dl	117.7 ± 34.5	130.2 ± 37.4	<0.001

CAD, coronary artery disease; DS, diameter stenosis.

Analyzing the data according to the extent of disease (no disease, mild disease, and one-, two-, or three-vessel disease) showed similar results (**Table 3**).

The association between OxLDL measures and obstructive CAD was also assessed using logistic regression analysis. Compared with the lowest quartile, the highest quartiles of all three types of IgM OxLDL autoantibodies and IgM apoB-ICs were associated with significantly or nearly significantly decreased odds ratio (OR) for obstructive CAD (**Table 4**), ranging in OR from 0.51 to 0.63. In contrast, no such relationships were noted with IgG OxLDL autoantibodies and apoB-ICs, except for a trend for IgG Cu-OxLDL autoantibodies (OR, 1.54; $P = 0.07$).

Combined effects of OxLDL autoantibodies and apoB-ICs and hypercholesterolemia on obstructive CAD

These relationships were further evaluated by determining ORs for obstructive CAD in patients with and without hypercholesterolemia using the lowest quartiles of OxLDL markers without hypercholesterolemia as the reference point. In the absence of hypercholesterolemia, the OR for obstructive CAD decreased progressively with increasing levels of IgM MDA-LDL autoantibodies and IgM apoB-IC (**Fig. 1A**). In the presence of hypercholesterolemia, quartile 1 of all IgM autoantibodies and apoB-ICs had an OR of ~3.3 for CAD, consistent with a strong association between hypercholesterolemia and obstructive CAD. However, the OR decreased progressively with increasing levels of IgM autoantibodies and apoB-ICs. In contrast, the OR for obstructive CAD tended to increase progressively with increasing levels of IgG OxLDL autoantibody and IgG apoB-ICs, particularly Cu-OxLDL (Fig. 1B).

Correlation between OxLDL measures and clinical and laboratory variables

In general, IgM autoantibodies and IgM apoB-ICs were modestly inversely correlated with age, serum creatinine, and homocysteine but positively associated with HDL-C (**Table 5**). No correlations were noted between hsCRP and any IgM and IgG OxLDL autoantibodies, except for a weak inverse correlation with OxCL IgG ($R = -0.09$, $P = 0.04$). Weak but statistically significant correlations were noted between OxLDL-E06 and IgG MDA-LDL ($R = 0.10$, $P = 0.021$), IgG apoB-IC ($R = 0.12$, $P = 0.009$), IgM apoB-IC ($R = 0.10$, $P = 0.026$), IgG Cu-OxLDL ($R = 0.12$, $P = 0.008$), and IgM Cu-OxLDL ($R = 0.09$, $P = 0.041$). There were also no statistically significant correlations between any OxLDL autoantibodies and apoB-ICs with total cholesterol, triglycerides, fibrinogen, or lipoprotein-associated lipoprotein lipase A_2.

TABLE 2. Association between OxLDL autoantibodies and apoB-IC levels and the presence of CAD

Parameter	No/Mild CAD (n = 233)	Obstructive CAD (n = 271)	*P*	No CAD (n = 122)	Any CAD (n = 382)	*P*
IgM						
MDA-LDL	16.1 (10.8, 23.0)	13.9 (9.3, 19.9)	0.016	16.7 (11.7, 23.5)	14.6 (9.6, 20.3)	0.010
Cu-OxLDL	5.2 (3.0, 7.6)	4.3 (2.8, 6.9)	0.037	5.5 (3.3, 7.8)	4.5 (2.8, 7.2)	0.019
OxCL	2.7 (1.7, 4.5)	2.2 (1.3, 3.7)	0.022	3.0 (1.8, 4.6)	2.3 (1.4, 3.9)	0.007
ApoB-IC	6.7 (4.0, 9.8)	5.5 (3.5, 8.5)	0.006	7.2 (4.3, 11.1)	5.7 (3.7, 8.5)	<0.001
IgG						
MDA-LDL	4.5 (3.4, 6.7)	4.6 (3.3, 6.8)	0.63	4.4 (3.3, 6.0)	4.7 (3.4, 6.9)	0.22
Cu-OxLDL	2.6 (1.7, 3.8)	2.9 (2.1, 4.1)	0.031	2.4 (1.7, 3.6)	2.8 (2.0, 4.2)	0.021
OxCL	0.8 (0.6, 1.1)	0.8 (0.6, 1.1)	0.57	0.8 (0.6, 1.1)	0.8 (0.6, 1.1)	0.95
ApoB-IC	5.0 (3.8, 6.9)	5.3 (3.9, 7.2)	0.10	5.0 (3.7, 6.9)	5.3 (3.9, 7.0)	0.17

ApoB-IC, apolipoprotein B-100 immune complex; Cu-OxLDL, copper oxidized low density lipoprotein; MDA, malondialdehyde; OxCL, oxidized cholesterol linoleate; OxLDL, oxidized low density lipoprotein. All OxLDL marker values are median and (interquartile range) and expressed as relative light units × 10^3. Obstructive CAD is defined as >50% DS.

TABLE 3. Association between OxLDL autoantibodies and apoB-IC levels and the extent of CAD

Parameter	None (n = 122)	Mild (n = 111)	One Vessel (n = 85)	Two Vessel (n = 80)	Three Vessel (n = 106)	*P*
IgM						
MDA-LDL	16.7 (11.7, 23.5)	15.4 (9.9, 21.2)	12.9 (8.7, 19.9)	14.4 (11.0, 20.0)	14.1 (9.1, 19.6)	0.027
Cu-OxLDL	5.5 (3.3, 7.8)	4.8 (2.8, 7.3)	3.7 (2.7, 6.9)	4.5 (3.0, 7.1)	4.5 (2.8, 6.8)	0.030
OxCL	3.0 (1.8, 4.6)	2.4 (1.6, 4.0)	2.5 (1.5, 3.6)	2.4 (1.5, 4.1)	2.1 (1.2, 4.0)	0.028
ApoB-IC	7.2 (4.3, 11.1)	6.0 (4.0, 8.4)	5.2 (3.2, 8.5)	5.5 (3.7, 8.2)	5.4 (3.4, 8.5)	0.017
IgG						
MDA-LDL	4.4 (3.3, 6.0)	5.1 (3.4, 7.2)	4.4 (3.3, 5.9)	4.4 (3.2, 7.1)	4.8 (3.7, 7.0)	0.18
Cu-OxLDL	2.4 (1.7, 3.6)	2.7 (1.9, 4.2)	2.8 (1.9, 3.8)	2.6 (2.0, 3.8)	3.1 (2.2, 4.5)	0.010
OxCL	0.8 (0.6, 1.1)	0.7 (0.6, 1.1)	0.7 (0.6, 1.2)	0.8 (0.6, 1.2)	0.8 (0.6, 1.1)	0.28
ApoB-IC	5.0 (3.6, 6.9)	5.1 (3.9, 6.8)	5.2 (3.7, 7.0)	5.1 (3.8, 7.1)	5.7 (4.2, 7.3)	0.027

All OxLDL marker values are in relative light units $\times 10^3$.

Correlations among OxLDL autoantibodies and apoB-ICs

There were strong correlations among all IgG classes of OxLDL autoantibodies and apoB-ICs as well as among all classes of IgM autoantibodies and IgM apoB-ICs. Less strong correlations were noted between IgG and IgM classes (**Table 6**).

Multivariable analysis

In multivariable analysis, age, LDL-C, smoking history, and hypertension were independent predictors of obstructive CAD, whereas female gender and HDL-C were independently associated with reduced risk of obstructive CAD (**Fig. 2**). There was no significant association between OxLDL measures and cardiovascular events in patients <60 or >60 years old. None of the IgG or IgM OxLDL measures or hsCRP was an independent predictor of obstructive CAD. There was no significant relationship between the OxLDL autoantibodies or apoB-IC variables and follow-up events after adjustment for covariates. **Table 7** shows the event rates at years 1, 2, and 3 for each OxLDL variable above and below its respective median value and documents no independent association of these markers at each time point. In addition, there was no association based on whether patients were categorized as none/mild CAD versus obstructive CAD (>50% DS).

DISCUSSION

This study assessed the relationship between both IgG and IgM OxLDL markers and angiographically determined CAD. In univariate analysis, a positive relationship of angiographically determined CAD with IgG Cu-OxLDL was present, whereas an inverse relationship existed between IgM OxLDL autoantibodies and IgM apoB-ICs, with ~40–50% reduction in the probability of the presence of >50% DS. However, in multivariable analysis taking into account other traditional risk factors, these OxLDL measures were no longer independent predictors of the presence of CAD or future cardiovascular events. This study provides a mechanistic framework to understand the disparate findings of previous studies by showing in a large population with robust statistical analysis that these OxLDL markers are univariate predictors of cardiovascular disease but, when viewed in the broad context of additional risk factors, likely play a secondary role rather than directly influencing CAD.

In contrast to the current study, we have shown from the same data set that circulating OxLDL-E06 levels, reflecting minimally oxidized LDL in the circulation, as opposed to OxLDL autoantibodies and apoB-ICs, which are indirect OxLDL markers, strongly and independently predicted the presence of angiographically determined

TABLE 4. Odds ratios and (95% confidence intervals) for obstructive CAD (>50% DS) according to quartiles of IgM and IgG OxLDL markers

	Quartile				
Patient Group	I	II	III	IV	*P* for Trend
IgM					
MDA-LDL	1.00	0.80 (0.68, 0.94)	0.64 (0.46, 0.87)	0.51 (0.32, 0.82)	0.005
Cu-OxLDL	1.00	0.86 (0.73, 1.00)	0.73 (0.53, 1.01)	0.63 (0.39, 1.01)	0.05
OxCL	1.00	0.86 (0.73, 1.00)	0.73 (0.54, 1.01)	0.63 (0.39, 1.01)	0.05
ApoB-IC	1.00	0.82 (0.70, 0.96)	0.67 (0.49, 0.92)	0.55 (0.34, 0.88)	0.013
IgG					
MDA-LDL	1.00	0.99 (0.84, 1.16)	0.97 (0.71, 1.33)	0.96 (0.60, 1.54)	0.87
Cu-OxLDL	1.00	1.16 (0.99, 1.35)	1.34 (0.97, 1.83)	1.54 (0.96, 2.48)	0.07
OxCL	1.00	1.04 (0.89, 1.21)	1.08 (0.79, 1.48)	1.12 (0.70, 1.79)	0.64
ApoB-IC	1.00	1.13 (0.96, 1.32)	1.27 (0.93, 1.74)	1.43 (0.89, 2.29)	0.14

Cutoff values (relative light units) for each quartile are as follows: IgM MDA-LDL (I, <9,930; II, 9,930–15,010; III, 15,010–21,270; IV, >21,270), IgM Cu-OxLDL (I, <2,933; II, 2,933–4,608; III, 4,608–7,331; IV, >7,331), IgM OxCL (I, <1,498; II, 1,498–2,491; III, 2,491–4,062; IV, >4,062), IgM apoB-IC (I, <3,775; II, 3,775–5,961; III, 5,961–9003; IV, >9,003); IgG MDA-LDL (I, <3,375; II, 3,375–4,535; III, 4,535–6,662; IV, >6,662), IgG Cu-OxLDL (I, <1,949; II, 1,949–2,747; III, 2,747–4,047; IV, >4,047), IgM OxCL (I, <617; II, 617–787; III, 787–1,135; IV, >1,135), IgM apoB-IC (I, <3,867; II, 3,867–5,188; III, 5,188–9,661; IV, >6,991).

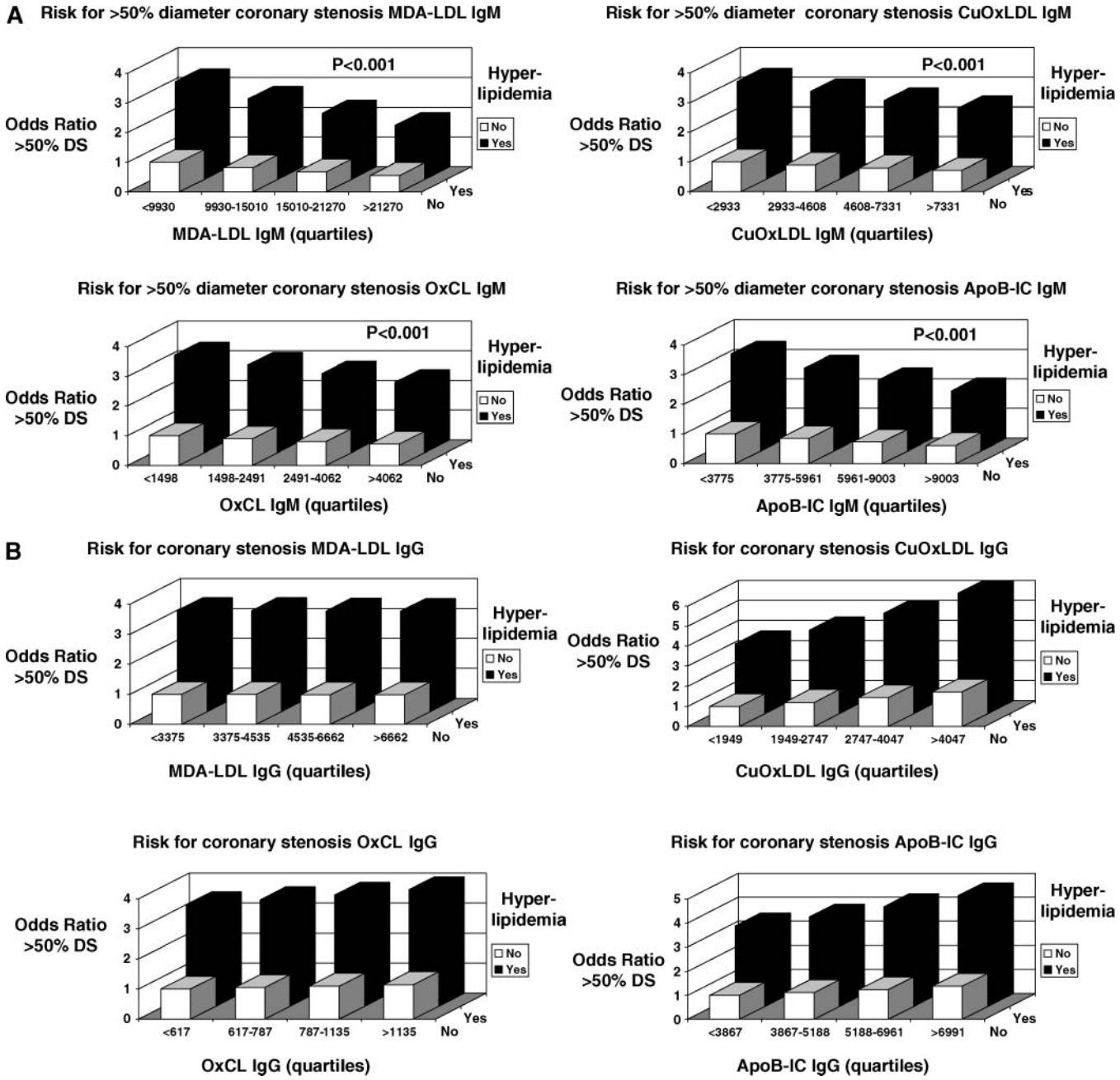

Fig. 1. Odds ratio (OR) for the presence of >50% diameter stenosis (DS) for IgM (A) and IgG (B) autoantibodies to oxidized low density lipoprotein (OxLDL) and apolipoprotein B-100 immune complexes (apoB-ICs) in the presence or absence of hypercholesterolemia. Cu-OxLDL, copper oxidized low density lipoprotein; MDA, malondialdehyde; OxCL, oxidized cholesterol linoleate.

CAD (13). This is consistent with a prior study showing that IgG and IgM autoantibodies to OxLDL were univariate but not independent predictors of carotid intima-media thickness (18), as opposed to OxLDL itself, which was an independent predictor (19). This suggests that direct OxLDL measures, such as circulating OxLDL (20), are more potent predictors of CAD than are indirect measures, such as OxLDL autoantibodies and immune complexes.

There are relatively few studies prospectively evaluating the prognostic value of autoantibodies to OxLDL and apoB-ICs and CAD. In this study, baseline OxLDL autoantibodies and apoB-ICs did not predict follow-up events, which occurred in 70 of 504 subjects followed over a median 4 year period. Previous studies evaluating clinical events suggested that IgG autoantibodies predicted the risk of future myocardial infarction (21). However, this has not been supported by all studies (12, 22). Our group showed previously that increased baseline levels of IgM apoB-ICs predicted a reduced risk of recurrent cardiovascular events over 4 months in patients with acute coronary syndromes in the Myocardial Ischemia Reduction with

TABLE 5. Spearman correlation rank coefficients between OxLDL autoantibodies and apoB-ICs and clinical and laboratory variables

Parameter	Age	Creatinine	LDL Cholesterol	HDL Cholesterol	Homocysteine	C-Reactive Protein
IgM						
MDA-LDL	−0.13 (0.003)	−0.14 (0.002)	−0.02 (0.72)	0.10 (0.03)	−0.10 (0.03)	0.08 (0.09)
Cu-OxLDL	−0.19 (<0.001)	−0.11 (0.01)	−0.08 (0.06)	0.11 (0.01)	−0.11 (0.010)	0.02 (0.69)
OxCL	−0.17 (<0.001)	−0.13 (0.003)	−0.03 (0.56)	0.11 (0.02)	−0.14 (0.002)	0.02 (0.58)
ApoB-IC	−0.27 (<0.001)	−0.12 (0.009)	−0.09 (0.04)	0.11 (0.01)	−0.14 (0.002)	−0.04 (0.41)
IgG						
MDA-LDL	0.16 (<0.001)	0.03 (0.46)	−0.01 (0.86)	0.003 (0.94)	0.08 (0.06)	0.004 (0.92)
Cu-OxLDL	0.13 (0.004)	0.07 (0.14)	0.02 (0.66)	−0.07 (0.11)	0.07 (0.12)	−0.03 (0.52)
OxCL	0.07 (0.11)	0.07 (0.11)	0.04 (0.36)	−0.05 (0.31)	0.12 (0.006)	−0.09 (0.04)
ApoB-IC	0.09 (0.04)	0.11 (0.02)	−0.04 (0.32)	−0.15 (<0.001)	0.09 (0.05)	−0.06 (0.20)

Values shown are *R* and (*P*).

Aggressive Cholesterol Lowering Study (17). The current data are also consistent with a previous clinical but not angiographic study showing that baseline IgM OxLDL autoantibody titers and IgM apoB-ICs were lowest in patients with acute myocardial infarction and unstable angina compared with normal subjects (16).

This is the first study to evaluate IgM OxLDL autoantibodies and immune complexes in angiographically determined CAD. Previous studies had suggested that IgM autoantibody titers to OxLDL are inversely associated with noncoronary atherosclerosis, noted primarily in Finnish patients (23). For example, Karvonen et al. (23) showed in a population-based cohort of 1,022 subjects that IgM MDA-LDL autoantibodies were independently inversely predictive of mean carotid intima-media thickness, although statistical significance was not reached in all parts of the carotid artery. That study also showed that IgG OxLDL autoantibodies were not independent predictors of carotid intima-media thickness. In a recent study from the Bruneck population, we confirmed that IgG OxLDL autoantibody titers and apoB-ICs were positively associated with carotid atherosclerosis, whereas IgM autoantibody titers and apoB-ICs were inversely related, but in univariate but not multivariate analysis (24). In that study, there was also a positive relationship of IgG OxLDL markers with age and male gender, whereas an inverse association was noted with IgM OxLDL markers. In a smaller study of 389 asymptomatic middle-aged males, IgM Cu-OxLDL autoantibody titers were borderline ($P = 0.05$) inversely associated with femoral intima-media thickness, but neither IgG nor IgM MDA-LDL autoantibody titers were associated with either carotid or femoral intima-media thickness (18). As in the current study, these relationships were no longer present after adjusting for traditional risk factors.

In this study, IgG Cu-OxLDL autoantibodies and apoB-IC levels were higher in patients with angiographically determined CAD but did not modulate atherogenesis or independently predict the presence of CAD. Although most studies have shown a positive association of IgG OxLDL autoantibodies with CAD, particularly smaller angiographic studies (25–27), others have shown no association (28–31). Several studies have also shown that IgG OxLDL autoantibodies are increased at baseline in patients with acute myocardial infarction (16, 32). In addition, we have also shown that circulating OxLDL, as well as both IgG and IgM autoantibodies to OxLDL, have a characteristic rise-and-fall pattern after myocardial infarction (16), which may be a confounding factor in some studies if the levels were not determined at the earliest time point possible. In addition, differences in antigen preparation and assay procedures, as well as inadequate sample size, may partially explain some of the findings (33). In this study, however, we performed a rigorous

TABLE 6. Spearman rank correlations (above diagonal) and *P* values (footnotes) among OxLDL autoantibodies and apoB-ICs

	IgG				IgM			
Parameter	MDA-LDL	Cu-OxLDL	OxCL	ApoB-IC	MDA-LDL	Cu-OxLDL	OxCL	ApoB-IC
IgG								
MDA-LDL	—	0.66[a]	0.54[a]	0.48[a]	0.14[b]	0.16[b]	0.16[b]	0.12[b]
Cu-OxLDL		—	0.50[a]	0.59[a]	0.08[c]	0.12	0.11[b]	0.05
OxCL			—	0.38[a]	0.16[a]	0.18[a]	0.27[a]	0.18[a]
ApoB-IC				—	0.02	0.04	0.03	0.03
IgM								
MDA-LDL					—	0.81[a]	0.77[a]	0.74[a]
Cu-OxLDL						—	0.77[a]	0.82[a]
OxCL							—	0.76[a]
ApoB-IC								—

[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.

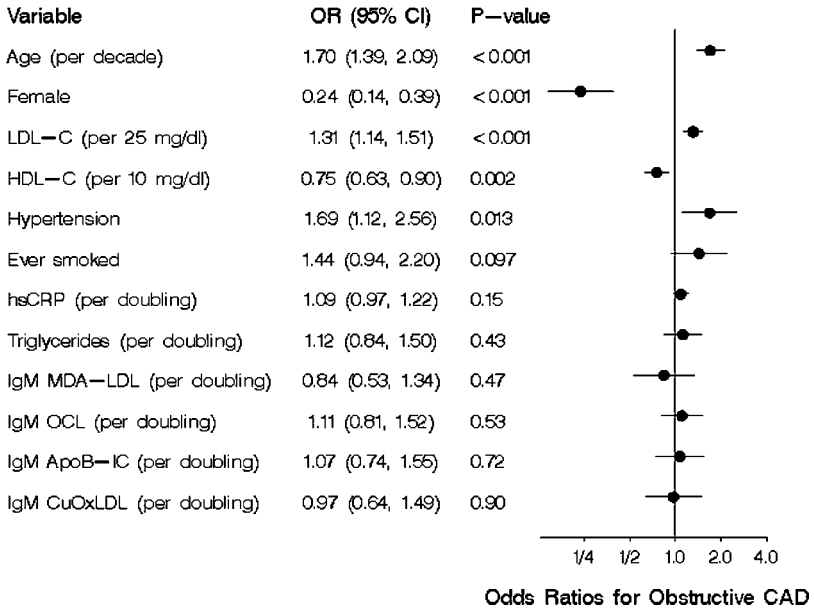

Fig. 2. Multivariable analysis depicting cardiovascular risk factors and OxLDL markers and the OR for the presence of >50% DS. HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; OCL, OxCL.

multivariable analysis with a large sample size, and the evidence suggests that IgG OxLDL autoantibodies do not predict angiographically determined CAD.

Why are IgM autoantibody titers lower in patients with manifestations of subclinical and symptomatic atherosclerosis and higher in patients without manifestations of atherosclerosis? One possibility is that in patients with risk factors for or with established CAD, there is increased consumption of IgM autoantibodies and ultimate clearance and/or uptake in the vessel wall. This is supported by data showing the presence of autoantibodies in the vessel wall (34) and by a previous study in our laboratory in which it was shown that in patients undergoing percutaneous coronary intervention, there was an acute decrease immediately after the procedure in both IgG and IgM OxLDL autoantibodies, with a simultaneous acute increase in apoB-ICs as well as increased OxLDL-E06 levels (35). It is possible that a similar situation occurs more slowly over time in patients with progressing atherosclerosis. Alternatively, there may be genetically determined differences in basal natural IgM autoantibody levels, and subjects with high levels of atheroprotective IgM autoantibodies may have less disease. Further research is required to understand why differences in basal levels of IgG and IgM autoantibodies to OxLDL exist and whether augmenting such titers above physiological levels may provide atheroprotection. For example, several groups have shown that immunization of rabbits and mice with autologous OxLDL or MDA-LDL provides atheroprotective effects (36–40). In addition, Binder et al. (41) subsequently showed that immunization of mice with *Streptococcus pneumoniae* results in a robust and predominant T15/E06 IgM autoantibody response and protection against atherosclerosis progression.

In conclusion, we have shown that OxLDL autoantibodies are not independent predictors of the presence of angiographically determined CAD or cardiovascular events. These findings need to be confirmed in studies with larger numbers of cardiovascular events measuring both IgG and IgM autoantibodies. Further studies are being carried out in several laboratories to assess whether

TABLE 7. Association between OxLDL autoantibodies and apoB-IC levels (above or below the median value) and the combined events of death, myocardial infarction, and stroke during the follow-up period

Parameter	1 Year	2 Years	3 Years	*P*
IgM MDA-LDL				
≤15,084	7 (3.0)	11 (4.7)	14 (6.0)	0.36
>15,084	7 (3.0)	11 (4.7)	14 (6.1)	
IgM Cu-OxLDL				
≤4,576	6 (2.6)	10 (4.3)	12 (5.2)	0.22
>4,576	8 (3.4)	12 (5.2)	16 (6.9)	
IgM OxCL				
≤2,452	7 (3.0)	11 (4.7)	13 (5.6)	0.68
>2,452	7 (3.0)	11 (4.8)	15 (6.5)	
IgM apoB-IC				
≤5,860	8 (3.4)	12 (5.1)	15 (6.4)	0.80
>5,860	6 (2.6)	10 (4.3)	13 (5.7)	
IgG MDA-LDL				
≤4,506	8 (3.4)	14 (6.0)	17 (7.3)	0.81
>4,506	6 (2.6)	8 (3.5)	11 (4.8)	
IgG Cu-OxLDL				
≤2,731	7 (3.0)	12 (5.1)	15 (6.4)	0.42
>2,731	7 (3.0)	10 (4.3)	13 (5.7)	
IgG OxCL				
≤780.8	5 (2.1)	12 (5.2)	14 (6.0)	0.90
>780.8	9 (3.9)	10 (4.3)	14 (6.1)	
IgG apoB-IC				
≤5,200	8 (3.4)	13 (5.6)	16 (6.8)	0.40
>5,200	6 (2.6)	9 (3.9)	12 (5.2)	
CAD				
None/mild	3 (1.5)	6 (3.0)	7 (3.5)	0.20
Obstructive	11 (4.2)	16 (6.1)	21 (8.1)	

OxLDL values are in relative light units and are presented as number (percent).

passive immunization with antibodies to OxLDL may lead to reduced atherosclerosis (12).

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