

Clinical applications of circulating oxidized low-density lipoprotein biomarkers in cardiovascular disease

Alexander E. Fraley and Sotirios Tsimikas

Purpose of review

The aim of this article is to review, analyze and interpret the growing body of evidence on circulating oxidized low-density lipoprotein and its relationship to diagnosis and prognosis of cardiovascular disease.

Recent findings

Previous studies focused on indirect measures of oxidative stress such as susceptibility of LDL to oxidation and measurement of autoantibodies to oxidized LDL. The generation of monoclonal antibodies recognizing distinct oxidation-specific epitopes has allowed the development of sensitive and specific assays to measure circulating oxidized LDL. Recent work in human populations has demonstrated that circulating oxidized LDL is associated with preclinical atherosclerosis, coronary and peripheral arterial atherosclerosis, acute coronary syndromes and vulnerable plaques. Several studies have also suggested that elevated levels of oxidized LDL are a prognostic indicator of cardiovascular outcomes. In addition, it has been shown that lipoprotein(a) is the primary carrier of oxidized phospholipids in the circulation of humans, suggesting additional mechanisms through which lipoprotein(a) may be pro-atherogenic.

Summary

Research on circulating oxidized LDL biomarkers is rapidly accelerating and providing novel insights into the pathophysiology of cardiovascular disease. Future studies will further assess the clinical utility of oxidized LDL biomarkers by determining their prognostic value in the diagnosis and prognosis of cardiovascular disease and will also evaluate the relative merit of specific assays by performing comparative studies.

Keywords

biomarkers, hypercholesterolemia, inflammation, low-density lipoprotein, oxidation, oxidative stress

Abbreviations

| | |
|--------------|----------------------------------|
| ACS | acute coronary syndromes |
| apoB | apolipoprotein B |
| CAD | coronary artery disease |
| IMT | intima-media thickness |
| MDA | malondialdehyde |
| OxLDL | oxidized low-density lipoprotein |
| OxPL | oxidized phospholipid |
| PCI | percutaneous intervention |

© 2006 Lippincott Williams & Wilkins
0957-9672

Introduction

A vigorous scientific effort is currently underway to develop blood biomarkers to diagnose cardiovascular disease and predict clinical outcomes. Plasma biomarkers have the advantage of being noninvasive, which makes them applicable to a greater proportion of patients at risk and, if abnormal, to allow opportunities to intervene at an earlier stage of disease. The ideal biomarker in atherosclerotic disease should reflect and quantify the extent of atherosclerotic burden, have accurate and reliable laboratory methods, provide good sensitivity and specificity, predict disease in asymptomatic individuals and be available for widespread application [1]. C-reactive protein most closely fulfills these criteria [2^{••}], yet it has not found universal acceptance, perhaps due to its non-specific nature, modest predictive value in individual patients compared to large epidemiologic studies, and the close correlation with the metabolic syndrome and other cardiovascular risk factors. Therefore, additional and more specific biomarkers will likely be useful in the diagnosis and prognosis of cardiovascular disease.

Atherosclerosis results from a combination of abnormalities in lipoprotein metabolism, oxidative stress, chronic inflammation and susceptibility to thrombosis. All of these processes contribute individually, or more commonly in aggregate, in the clinical expression of cardiovascular disease [3,4]. Oxidized low-density lipoprotein (OxLDL) is pro-inflammatory and pro-atherogenic and is intimately involved in the initiation, progression and potentially in the destabilization of atherosclerotic lesions [5]. Therefore, it may be a unifying factor that can influence underlying risk in lipid disorders, inflammation and thrombosis. Direct visualization of OxLDL in the vessel wall would be ideal to measure such risk, and although an area of active study [6,7], is not currently feasible in humans. The use of OxLDL biomarkers, however, shows promise

Curr Opin Lipidol 17:502–509. © 2006 Lippincott Williams & Wilkins.

From the Division of Cardiology, University of California, San Diego, California, USA

Correspondence to Sotirios Tsimikas, MD, Vascular Medicine Program, University of California San Diego, 9350 Campus Point Drive, Cardiovascular Diseases, La Jolla, CA 92037-0975, USA.
E-mail: stsimikas@ucsd.edu

Current Opinion in Lipidology 2006, 17:502–509

in diagnosing preclinical atherosclerosis in asymptomatic individuals, monitoring active disease and predicting cardiovascular outcomes. This review will summarize the recent literature on circulating OxLDL biomarkers with an emphasis on the clinical application of these assays.

Measures of circulating oxidized LDL

The term OxLDL was traditionally used to describe LDL modified by exposure to copper ions, which catalyzed lipid peroxidation. The term OxLDL in a generic sense, however, additionally describes a broad array of chemical, biological and immunological entities, ranging from measurement of conjugated dienes, susceptibility of LDL to oxidation, apolipoprotein B (apoB)-immune complexes and autoantibodies to various epitopes of OxLDL. Many of these assays are nonspecific for LDL oxidation, are imprecise, have poor specificity and are not adaptable to large-scale studies. This has led to difficulty or inability to compare or interpret results in different studies and has engendered significant controversy as to the pathophysiological role and clinical relevance of these measures [8].

The development of monoclonal antibodies binding oxidation-specific epitopes has allowed the development of sensitive and specific assays to measure circulating OxLDL [9–11]. Figure 1 displays the methodology for three well described OxLDL assays from which the majority of published clinical data exists. The three assays are not necessarily comparable as they either use antibodies detecting different epitopes or are set up in different formats and units of measurement. To minimize confusion and to allow more appropriate comparisons among studies, we have previously suggested that investigators describe their measures of OxLDL by

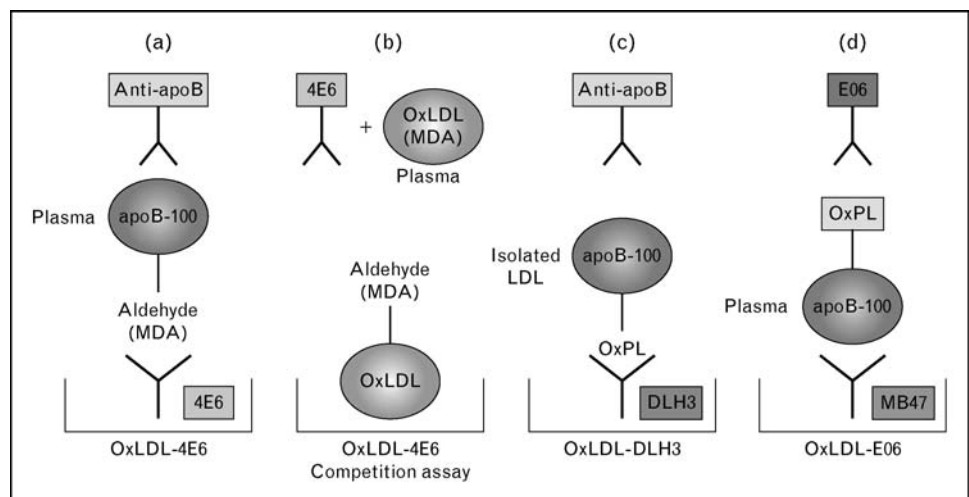
linking their antibody to the type of OxLDL described, (i.e. 'OxLDL-E06' for the E06 antibody) [12].

The murine monoclonal antibody 4E6, developed by immunizing Balb/c mice with copper-oxidized LDL, binds to aldehyde-modified lysine groups [likely malondialdehyde (MDA)-lysine epitopes] on LDL (MDA-LDL), although it does cross react also with copper-oxidized LDL [13,14]. OxLDL-4E6 is available commercially as a sandwich enzyme-linked immunosorbent assay in two formats for research purposes (Mercodia, Inc, Uppsala, Sweden). The traditional assay captures OxLDL from plasma with antibody 4E6 attached to microtiter plates and detects the content of apoB-100 with a secondary antibody (Fig. 1a). The competitive assay described recently (Fig. 1b) plates a 'standard' human OxLDL preparation to microtiter wells and adds the plasma sample together with biotinylated 4E6. Reduced binding of 4E6 to the plate implies higher plasma OxLDL levels. This version of the assay is theoretically more sensitive to the number of oxidation-specific epitopes on each LDL particle than the standard version. Most of the published data are with the standard assay and it has not been shown whether the competitive assay will yield similar results since it is fundamentally a different methodology. Studies comparing the two assays are not available and interpretation of the literature from this particular assay should keep this in mind. The results are presented as units per liter based on a standard curve that employs the reference OxLDL.

The murine IgM monoclonal antibody DLH3/FOH1a (OxLDL-DLH3) recognizes oxidized phosphocholine epitopes on LDL and was generated by immunizing mice with human coronary atheroma [15]. The OxLDL-DLH3 assay (Fig. 1c) is set up in a similar manner to the

Figure 1 Description of the methodology of the three major oxidized LDL assays

(a,b) Standard and competition version of the oxidized LDL (OxLDL)-4E6 assay. (c) The OxLDL-DLH3 assay. (d) The OxLDL-E06 assay. 4E6, DLH3, and E06 are oxidation-specific antibodies. MB47 is a murine monoclonal antibody binding human apolipoprotein B (apoB)-100. MDA, malondialdehyde; OxPL, oxidized phospholipids. Reproduced from S. Tsimikas, *Clinics in Laboratory Medicine*; published by Elsevier, 2006.



OxLDL-4E6 assay, except that it uses isolated LDL rather than plasma to measure OxLDL. This presumably reduces interference from epitopes in plasma not on LDL, but also makes it a labor-intensive procedure not amenable to high-throughput or large studies. The assay results are presented in units per milliliter, where one unit equals the absorbance of 250 ng of the reference OxLDL.

The IgM monoclonal antibody E06 (OxLDL-E06) recognizes the phosphocholine headgroup of oxidized phospholipids (OxPLs) but not normal phospholipids [10,16]. The OxLDL-E06 assay is set up in a fundamentally different manner to the previous two assays described above by capturing an equal and minute proportion of plasma apoB-100 in all microtiter wells from each subject with murine monoclonal antibody MB47, irrespective of the plasma apoB-100 level (Fig. 1d). Thus, the assay is independent of LDL-cholesterol or apoB levels. OxPL on apoB-100 are then detected with biotinylated E06 to determine the OxPL/apoB ratio and presented as relative light units (RLUs) per 100 ms. This assay is highly specific to the number of OxPL epitopes on individual apoB-100 particles.

The OxLDL-4E6 assay is standardized and commercially available but the epitopes it binds are not precisely known and a strong correlation (approximately 0.65–0.70) exists between OxLDL-4E6 and LDL-C. The close correlation with LDL-C is not expected as OxLDL and LDL have markedly different kinetics of formation and clearance, and may represent methodological issues, such as the lack of sensitivity to the number of epitopes or other factors. The OxLDL-DLH3 assay detects pro-inflammatory oxidized phosphocholine but requires isolated LDL, which limits widespread use. Both assays require a calibration reference OxLDL be made batch to batch from human donors, which may potentially lead to different reference scales. The OxLDL-E06 assay format is not currently standardized to a reference OxPL, but instead uses a relative scale, which does not allow ease of comparison among different studies. Plasma levels of OxPL/apoB also strongly correlate with lipoprotein(a) (r value, approximately 0.85), but not with other lipoproteins. This was determined to be due to the surprising observation that, in humans, lipoprotein(a) particles rather than non-lipoprotein(a)-apoB-100 particles carry most of OxPL [17–20,21^{••},22,23^{••}]. This property has provided significant insights into the atherogenicity of lipoprotein(a) in binding and transporting OxPL. The OxLDL-DLH3 and OxLDL-E06 assays are not commercially available.

In summary, all of the above assays have some advantages and disadvantages currently and comparative data are awaited to assess if any one provides enhanced clinical utility compared with the others. None of these assays is currently approved for routine clinical use.

OxLDL as a marker of asymptomatic cardiovascular disease

Most, if not all, cardiovascular risk factors generate oxidative stress in the vessel wall. As LDL traverses the subendothelial space it becomes oxidized, prior to advanced lesion formation, and may induce endothelial dysfunction, one of the earliest manifestations of atherosclerosis. In-vitro and in-vivo studies have shown that OxLDL promotes endothelial cell toxicity and vasoconstriction. OxLDL levels correlate with endothelial dysfunction and with improvement following lipid-lowering therapy with apheresis or statins (reviewed by Navab *et al.* [24]). Plasma levels of OxLDL-DLH3 were recently shown to be an independent determinant of coronary macrovasomotor and microvasomotor responses in response to bradykinin in humans [25].

The relationship of OxLDL to cardiovascular risk factors is not fully established. Because most of OxLDL is present in the vessel wall rather than plasma (100-fold greater levels compared to plasma [26]), plasma levels may not necessarily reflect an association with all risk factors. Nonetheless, OxLDL levels have been associated with small dense LDL [27,28] and the metabolic syndrome [29] using the OxLDL-4E6 assay, although conflicting results are present as a second cohort found no association using the same assay, even after adjusting for LDL-cholesterol levels [30]. As discussed below, the OxLDL-4E6 correlates with LDL-cholesterol and OxLDL-E06 (OxPL/apoB) and OxLDL-DLH3 with lipoprotein(a) ($r=0.77$) [31]. OxPL/apoB and lipoprotein(a) lack correlations with most cardiovascular risk factors, including C-reactive protein. Further research is required to formally assess the relationship of OxLDL measures to cardiovascular risk factors in large cohorts of patients.

The value of OxLDL in screening selected high-risk subjects was demonstrated in a study of asymptomatic family member of subjects with familial hyperlipidemia [32]. In these individuals elevated OxLDL-4E6 and LDL-cholesterol levels were associated with increased carotid intima-media thickness (IMT). This association was further investigated in a population study of middle-aged males from the general population in whom OxLDL-4E6 predicted carotid and femoral IMT, although with borderline statistical significance [33]. Interestingly, in a larger population with heterozygous familial hypercholesterolemia, OxLDL-4E6 did not predict IMT, either at baseline or following statin therapy [34].

Association of oxidized LDL with coronary artery disease

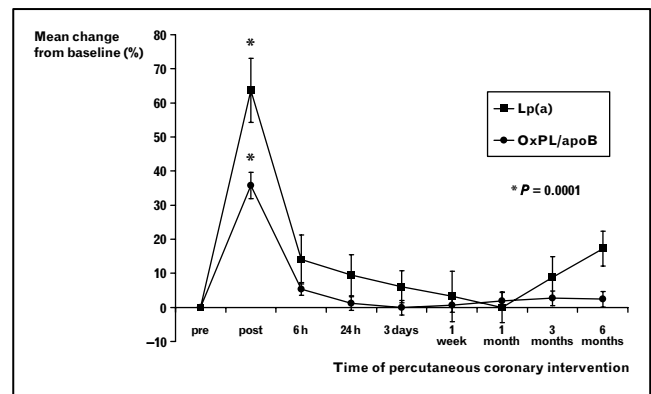
In a recent study evaluating a population of 504 patients undergoing coronary angiography for stable coronary artery disease (CAD), OxLDL-E06 (OxPL/apoB) levels correlated with both the presence and extent of

angiographically-determined CAD [21**]. Patients in the highest quartile of circulating OxLDL-E06 had the most advanced angiographically detected CAD with three-vessel involvement. Interestingly, lipoprotein(a) had a strikingly similar association with CAD and a strong correlation ($r=0.83$, $P<0.001$) was noted between OxPL/apoB and lipoprotein(a), suggesting a common biological influence on cardiovascular risk. In logistic regression analysis, compared to the lowest quartile, subjects in the highest quartile had an odds ratio of 1.95 for greater than 50% diameter stenosis in any coronary artery. Patients under 60 years with coexisting hypercholesterolemia, however, had an odds ratio of 16.8. In the overall cohort, this association was independent of all clinical and lipid risk factors (including C-reactive protein), except for lipoprotein(a). In patients under 60 years old, however, OxPL/apoB levels were independent even of lipoprotein(a) suggesting that OxPL may mediate atherogenicity above and beyond its association with lipoprotein(a), perhaps through additional pro-inflammatory mechanisms. The influence of OxPL and lipoprotein(a) on CAD risk was diminished in patients over 60 years old, perhaps through the accumulation of additional risk factors that may have superseded the risk of OxPL/apoB and lipoprotein(a), which can be elevated at birth in many subjects [22]. The association of OxLDL with stable CAD has also been confirmed using the OxLDL-DLH3 and OxLDL-4E6 assays in previous studies [27,35–37].

Association of oxidized LDL with acute coronary syndromes and percutaneous coronary intervention, vulnerable plaques and restenosis

The association of OxLDL with acute coronary syndromes (ACSs) was previously documented in cross-sectional studies, generally showing higher levels of OxLDL according to the severity of presentation [38–40]. In addition, endothelial derived soluble LOX-1 receptor levels are also elevated in ACS [41]. In a prospective study, it was recently demonstrated that OxLDL-E06 levels rise rapidly in patients suffering acute myocardial infarction and then tend to decrease toward baseline levels over the next 7 months [17]. This has implications regarding generation of OxLDL during ACS, release of OxLDL from atherosclerotic plaques and interpretation of studies in which OxLDL levels are measured in unstable patients which levels may not reflect baseline values. In a follow-up study, it was demonstrated that OxPL/apoB and lipoprotein(a) levels also acutely increase immediately following percutaneous intervention (PCI) (Fig. 2), further supporting the hypothesis that plaque disruption, iatrogenically induced in this case, results in release of OxPL from the vessel wall [19]. Interestingly, the OxPL/apoB levels returned to baseline by 6 h and precipitation experiments showed that immediately after PCI, approximately 50% of

Figure 2 Mean percentage change in oxidized phospholipids/apolipoprotein B and lipoprotein(a) levels before, immediately following and over the next 6 months in patient with stable angina undergoing elective percutaneous coronary intervention



Note the significant rise in oxidized phospholipids/apolipoprotein B (OxPL/apoB) and lipoprotein (Lp(a)) immediately following percutaneous coronary intervention (approximate duration of procedure is 1 h) with return to baseline levels by 6 h after the procedure. Reproduced with permission [19].

OxPLs were present on lipoprotein(a) whereas the other 50% were present on non-lipoprotein(a) apoB-100 particles. By 6 h after PCI however, more than 90% of OxPL were again present on lipoprotein(a), suggesting that, when present, lipoprotein(a) preferentially binds and transports mobilized OxPL. In this study, it was also shown that there was an immediate fall in preexisting autoantibody levels to MDA-LDL and an acute rise in apoB-immune complexes and subsequently returning back to baseline, further suggesting that release of OxLDL results in immune complex formation with ultimate clearance of these particles [19].

Nishi *et al.* [26] showed that ‘vulnerable’ carotid plaques, documented as such with accepted pathological criteria following carotid endarterectomy, were highly enriched in macrophages and OxLDL (with approximately 100-fold greater levels in lesions than plasma) compared to stable carotid plaques. It has also been shown that a 3-month treatment with pravastatin prior to carotid endarterectomy significantly reduces OxLDL content in carotid plaques [42]. Similarly, OxLDL content by immunostaining was also higher in coronary atherectomy specimens in patient with ACS [38]. It was also shown that monocytes derived from patients with unstable angina induced a significant amount of nuclear factor- κ B when exposed to patient’s serum with elevated OxLDL levels [43*]. Furthermore, OxLDL levels correlate with angiographically complex plaques [44,45]. Overall, these data make a strong argument that OxLDL biomarkers strongly reflect the presence of ACS and that OxLDL may be integrally involved in plaque destabilization.

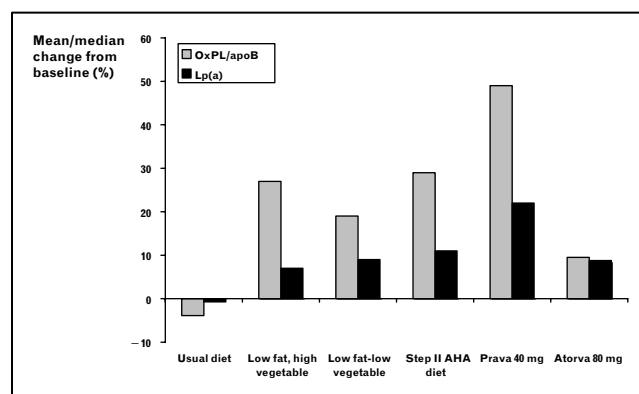
Restenosis is now less frequent with drug-eluting stents but additional predictors are still needed to risk-stratify patients. Segev *et al.* [45] showed that OxLDL-E06 levels, despite increasing after PCI, were not associated with restenosis at 6-month angiographic follow-up in 135 patients undergoing uncomplicated PCI for stable angina. Naruko *et al.* [46], however, showed in 102 consecutive patients with acute myocardial infarction that if OxLDL-DLH3 levels remained elevated prior to hospital discharge, they predicted subsequent restenosis. A third study of 687 patients with a heterogeneous population that included postmyocardial infarction, stable and unstable angina patients, however, showed that OxLDL-4E6 levels did not predict the incidence of death, myocardial infarction, target vessel revascularization (the combined primary end-point) or angiographically determined restenosis [47]. Thus, it appears that OxLDL may not have a significant role in predicting restenosis or outcomes following coronary stenting.

The effect of diet and medical therapy on oxidized LDL

The impact of diet and statin therapy on OxLDL levels is currently just starting to be explored. Several cross-sectional and prospective studies have recently shown either that OxLDL levels, measured with the OxLDL-4E6 and OxLDL-DLH3 assays as well as by MDA-LDL levels with a different assay, are lower in patients on statins or fall in patients treated with a variety of statins, including atorvastatin, simvastatin, pravastatin, and fluvastatin [27,34,48–50].

Interestingly, the OxLDL-E06 assay, which determines the content of OxPL per apoB particle rather than total levels of OxLDL in plasma and is very sensitive to net content of OxPL epitopes on apoB-100 compared to the other assays, gives different and seemingly counterintuitive information (Fig. 3). In a study of the response of OxLDL-E06 to two low-fat diets, Silaste *et al.* [51] showed that median OxPL/apoB levels increased by 27% ($P < 0.01$) in response to the low-fat, low-vegetable diet and 19% ($P < 0.01$) in response to the low-fat, high-vegetable diet, despite no change in plasma apoB levels. Interestingly, the change in lipoprotein(a) concentration mirrored the change in OxPL/apoB levels. Similar results with a mean 29.3% increase have been reported with a step II AHA diet in children with familial hypercholesterolemia [22]. Additionally, increases in the OxPL/apoB ratio were also documented prospectively in 2341 patients treated with atorvastatin 80 mg/day versus placebo (median 9.5% increase in atorvastatin but no change in placebo [20]) and pravastatin (median 48.7% increase [22]). Although such increases in the OxPL/apoB ratio remain unexplained at present, we previously hypothesized that mobilization of OxPL may occur from atherosclerotic lesions, sites of inflammation, or even via

Figure 3 Mean or median percentage change in oxidized phospholipids/apolipoprotein B and lipoprotein(a) levels in response to diet or statin therapy from baseline to follow-up in several prospective studies



Oxidized phospholipids/apolipoprotein B (OxPL/apoB) and lipoprotein(a) (Lp(a)) levels rise in all cases except in patients on placebo treatment.

transfer from other lipoproteins to OxPL acceptors, such as lipoprotein(a), which strongly binds OxPL [17–20,21**,22,23**]. Additional unpublished data from our laboratory show that animals without lipoprotein(a) (rabbits) or those whose lipoprotein(a) does not bind OxPL (cynomolgous monkeys) also have increases in the OxPL/apoB ratio with concomitant disappearance of OxPL from the vessel wall. This implies that OxPL may egress out of the vessel wall and into the circulation during dietary or statin intervention and suggests a causal relationship between increased OxPL/apoB ratio and early atherosclerosis regression or plaque stabilization.

Association of oxidized LDL with prognosis of cardiovascular disease

The utility of OxLDL-E06 in predicting progression of atherosclerosis was recently evaluated in the Bruneck study, a large prospective population-based survey of 40–79-year-old men and women initiated in 1990 [23**]. Serial plasma levels of OxPL/apoB and lipoprotein(a) were acquired in 765 of 826 (92.6%) and 671 of 684 (98.1%) subjects in 1995 and 2000, respectively. The cohort was evaluated for baseline cardiovascular events and also followed for progression of atherosclerosis with serial measurement of femoral and carotid IMT. The OxPL/apoB distribution was clustered at the lower end of the scale [as known previously for Lp(a) levels in populations] and remained highly correlated with Lp(a) ($r = 0.87$, $P < 0.001$) and this correlation was strongest in individuals with the highest concentrations of Lp(a) and fewest number of kringle IV-2 repeats. Individual paired OxPL/apoB samples from 1995 and 2000 were highly correlated ($r = 0.78$, $P < 0.001$), displaying a better correlation than LDL-cholesterol ($r = 0.62$, $P < 0.001$) or HDL-cholesterol over the 5-year period. Both OxPL/apoB and lipoprotein(a) lacked correlations with most

cardiovascular risk factors and life-style variables. Interestingly, the number of apolipoprotein(a) kringle IV-2 repeats was inversely related to lipoprotein(a) mass ($r = -0.48$; $P < 0.001$) and OxPL/apoB levels ($r = -0.46$; $P < 0.001$). In multivariable analysis, OxPL/apoB levels were strongly and significantly associated with the presence, extent and interim development of carotid and femoral atherosclerosis over the 5-year period. OxPL/apoB and lipoprotein(a) also predicted the presence of symptomatic cardiovascular disease at entry into the study. There were not enough accrued events over the 5-year follow-up period to determine if OxPL/apoB levels predicted new clinical events. Both OxLDL-EO6/apoB and lipoprotein(a) levels showed similar associations with atherosclerosis severity and progression, suggesting a common biological influence on atherogenesis and provided information beyond typical risk factors in both the diagnosis and monitoring of cardiovascular disease.

OxLDL is now being studied as a predictor of secondary cardiovascular events. For example, in a prospective study of 238 patients with known CAD followed for a mean of 52 months, baseline OxLDL-DLH3 levels were an independent predictor of cardiac death, non-fatal myocardial infarction and unstable angina, with patients in the highest quartile having a hazard ratio 3.15 times greater than patients in the lowest quartile [52]. In a study evaluating the prognostic value of OxLDL for CAD in a high-risk population with the metabolic syndrome, OxLDL-4E6 did not independently predict CAD, but did show that individuals with metabolic syndrome had increased risk for acute myocardial infarction (odds ratio 2.25; 1.22–4.15) compared to those without metabolic syndrome [29]. The predictive power of circulating OxLDL was more robust in a prospective, nested, case–control study with median 5.6-year follow-up showing that baseline OxLDL-4E6 levels, using the competitive assay, were higher in those subjects who subsequently had future cardiovascular events and that OxLDL was a stronger predictor of future events versus other lipoprotein measures [53^{••}]. This study was limited to men, however, and although multivariable analysis was performed adjusting for most cardiovascular risk factors, it is not clear if OxLDL-E06 levels were evaluated in a model including LDL-cholesterol to see if the results were independent of LDL-cholesterol, as there was a high correlation between OxLDL-E06 and LDL-cholesterol ($r = 0.69$; $P < 0.001$).

Conclusion

The field of OxLDL, in a bench to bedside transition, has progressed over the last 25 years from an in-vitro and cell culture science, to animal models of atherogenesis and now to plasma biomarkers. Although most

of the vitamin E antioxidant trials have failed to show a clinical benefit, for many reasons [54], the ability to now measure OxLDL and identify those patients with high oxidative stress prior to any therapeutic intervention will allow more rational and targeted approaches. From the current state-of-the-art, it can be concluded that circulating OxLDL levels correlate independently with various forms of coronary and peripheral arterial disease. Studies assessing the prognostic value of OxLDL are at their infancy and future large and rigorous prospective studies will attempt to answer the question of whether OxLDL biomarkers independently predict clinical outcomes. In particular, studies are needed to further establish the relationship of OxLDL levels to therapeutic interventions and the relationship to clinical outcomes. Since OxLDL represents divergent oxidative and immunological species, comparative studies of the various assays are also needed to assess which assays, assay formats and which oxidation-specific epitopes are most predictive of cardiovascular disease. The answers to these questions will allow us to decide whether and which OxLDL biomarkers can ultimately be integrated into routine clinical decision-making.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 591–596).

- 1 Manolio T. Novel risk markers and clinical practice. *N Engl J Med* 2003; 349:1587–1589.
- 2 Tsimikas S, Willerson JT, Ridker PM. C-Reactive protein and other emerging blood biomarkers to optimize risk stratification of vulnerable patients. *J Am Coll Cardiol* 2006; 47:C19–C31.
- This is a broad review of the field of blood biomarkers currently used in the contemporary management of patients and with particular emphasis on emerging biomarkers.
- 3 Steinberg D. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nat Med* 2002; 8:1211–1217.
- 4 Libby P. Act local, act global: Inflammation and the multiplicity of “vulnerable” coronary plaques. *J Am Coll Cardiol* 2005; 45:1600–1602.
- 5 Tsimikas S, Glass C, Steinberg D, *et al.* Lipoproteins, lipoprotein oxidation and atherogenesis. In: Chien KR, editor. *Molecular basis of cardiovascular disease: A companion to Braunwald’s heart disease*. Philadelphia: W.B. Saunders Company; 2004. pp. 385–413.
- 6 Torzewski M, Shaw PX, Han KR, *et al.* Reduced in vivo aortic uptake of radiolabeled oxidation-specific antibodies reflects changes in plaque composition consistent with plaque stabilization. *Arterioscler Thromb Vasc Biol* 2004; 24:2307–2312.
- 7 Tsimikas S, Palinski W, Halpern SE, *et al.* Radiolabeled MDA2, an oxidation-specific, monoclonal antibody, identifies native atherosclerotic lesions in vivo. *J Nucl Cardiol* 1999; 6:41–53.
- 8 Shoefeld Y, Wu R, Dearing LD, *et al.* Are antioxidant Low-Density Lipoprotein antibodies pathogenic or protective? *Circulation* 2004; 110:2552–2558.
- 9 Holvoet P, Perez G, Zhao Z, *et al.* Malondialdehyde-modified low density lipoproteins in patients with atherosclerotic disease. *J Clin Invest* 1995; 95:2611–2619.
- 10 Hörkkö S, Bird DA, Miller E, *et al.* Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. *J Clin Invest* 1999; 103:117–128.

- 11 Itabe H, Yamamoto H, Imanaka T, *et al.* Sensitive detection of oxidatively modified low density lipoprotein using a monoclonal antibody. *J Lipid Res* 1996; 37:45–53.
- 12 Tsimikas S, Witztum JL. Measuring circulating oxidized low-density lipoprotein to evaluate coronary risk. *Circulation* 2001; 103:1930–1932.
- 13 Holvoet P, Donck J, Landeloos M, *et al.* Correlation between oxidized low density lipoproteins and von Willebrand factor in chronic renal failure. *Thromb Haemost* 1996; 76:663–669.
- 14 Holvoet P, Van Cleemput J, Collen D, *et al.* Oxidized low density lipoprotein is a prognostic marker of transplant-associated coronary artery disease. *Arterioscler Thromb Vasc Biol* 2000; 20:698–702.
- 15 Itabe H, Takeshima E, Iwasaki H, *et al.* A monoclonal antibody against oxidized lipoprotein recognizes foam cells in atherosclerotic lesions. Complex formation of oxidized phosphatidylcholines and polypeptides. *J Biol Chem* 1994; 269:15274–15279.
- 16 Friedman P, Hörkö S, Steinberg D, *et al.* Correlation of antiphospholipid antibody recognition with the structure of synthetic oxidized phospholipids: Importance of Schiff base formation and Aldol condensation. *J Biol Chem* 2001; 277:7010–7020.
- 17 Tsimikas S, Bergmark C, Beyer RW, *et al.* Temporal increases in plasma markers of oxidized low-density lipoprotein strongly reflect the presence of acute coronary syndromes. *J Am Coll Cardiol* 2003; 41:360–370.
- 18 Edelstein C, Pfaffinger D, Hinman J, *et al.* Lysine-phosphatidylcholine adducts in kringle V impart unique immunological and potential pro-inflammatory properties to human apolipoprotein(a). *J Biol Chem* 2003; 278:52841–52847.
- 19 Tsimikas S, Lau HK, Han KR, *et al.* Percutaneous coronary intervention results in acute increases in oxidized phospholipids and lipoprotein(a): Short-term and long-term immunologic responses to oxidized low-density lipoprotein. *Circulation* 2004; 109:3164–3170.
- 20 Tsimikas S, Witztum JL, Miller ER, *et al.* High-dose atorvastatin reduces total plasma levels of oxidized phospholipids and immune complexes present on apolipoprotein B-100 in patients with acute coronary syndromes in the MIRACL trial. *Circulation* 2004; 110:1406–1412.
- 21 Tsimikas S, Brilakis ES, Miller ER, *et al.* Oxidized phospholipids, Lp(a) lipoprotein, and coronary artery disease. *N Engl J Med* 2005; 353:46–57.
- This study established that the OxPL/apoB ratio is an independent predictor of the presence and extent of angiographically determined coronary artery disease in patients under 60 years old.
- 22 Rodenburg J, Vissers MN, Wiegman A, *et al.* Oxidized low-density lipoprotein in children with familial hypercholesterolemia and unaffected siblings: Effect of pravastatin. *J Am Coll Cardiol* 2006; 47:1803–1810.
- 23 Tsimikas S, Kiechl S, Willeit J, *et al.* Oxidized phospholipids predict the presence and progression of carotid and femoral atherosclerosis and symptomatic cardiovascular disease: Five-year prospective results from the Bruneck Study. *J Am Coll Cardiol* 2006; 47:2219–2228.
- This was a large population-based study of unselected patients from the general community showing that OxPL/apoB levels predict the presence and progression of peripheral atherosclerotic disease.
- 24 Navab M, Anantharamaiah GM, Reddy ST, *et al.* Thematic review series: The pathogenesis of atherosclerosis: The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. *J Lipid Res* 2004; 45:993–1007.
- 25 Matsumoto T, Takashima H, Ohira N, *et al.* Plasma level of oxidized low-density lipoprotein is an independent determinant of coronary macrovasomotor and microvasomotor responses induced by bradykinin. *J Am Coll Cardiol* 2004; 44:451–457.
- 26 Nishi K, Itabe H, Uno M, *et al.* Oxidized LDL in carotid plaques and plasma associates with plaque instability. *Arterioscler Thromb Vasc Biol* 2002; 22:1649–1654.
- 27 Holvoet P, Harris TB, Tracy RP, *et al.* Association of high coronary heart disease risk status with circulating oxidized LDL in the well functioning elderly: Findings from the health, aging, and body composition study. *Arterioscler Thromb Vasc Biol* 2003; 23:1444–1448.
- 28 Tanaga K, Bujo H, Inoue M, *et al.* Increased circulating malondialdehyde-modified LDL levels in patients with coronary artery diseases and their association with peak sizes of LDL particles. *Arterioscler Thromb Vasc Biol* 2002; 22:662–666.
- 29 Holvoet P, Kritchevsky SB, Tracy RP, *et al.* The metabolic syndrome, circulating oxidized LDL, and risk of myocardial infarction in well functioning elderly people in the health, aging, and body composition cohort. *Diabetes* 2004; 53:1068–1073.
- 30 Sjogren P, Basu S, Rosell M, *et al.* Measures of oxidized low-density lipoprotein and oxidative stress are not related and not elevated in otherwise healthy men with the metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2005; 25:2580–2586.
- 31 Suzuki T, Kohno H, Toshima S, *et al.* Diagnostic implications of circulating oxidized low density lipoprotein levels as a biochemical risk marker of atherosclerotic disease. *Clin Biochem* 2002; 35:347–353.
- 32 Liu ML, Ylitalo K, Salonen R, *et al.* Circulating oxidized low-density lipoprotein and its association with carotid intima-media thickness in asymptomatic members of familial combined hyperlipidemia families. *Arterioscler Thromb Vasc Biol* 2004; 24:1492–1497.
- 33 Hulthe J, Fagerberg B. Circulating oxidized LDL is associated with subclinical atherosclerosis development and inflammatory cytokines (AIR Study). *Arterioscler Thromb Vasc Biol* 2002; 22:1162–1167.
- 34 van Tits LJH, van Himbergen TM, Lemmers HLM, *et al.* Proportion of oxidized LDL relative to plasma apolipoprotein B does not change during statin therapy in patients with heterozygous familial hypercholesterolemia. *Atherosclerosis* 2006; 185:307–312.
- 35 Holvoet P, Mertens A, Verhamme P, *et al.* Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 2001; 21:844–848.
- 36 Suzuki T, Kohno H, Hasegawa A, *et al.* Diagnostic implications of circulating oxidized low density lipoprotein levels as a biochemical risk marker of coronary artery disease. *Clin Biochem* 2002; 35:347–353.
- 37 Toshima S, Hasegawa A, Kurabayashi M, *et al.* Circulating oxidized low density lipoprotein levels: A biochemical risk marker for coronary heart disease. *Arterioscler Thromb Vasc Biol* 2000; 20:2243–2247.
- 38 Ehara S, Ueda M, Naruko T, *et al.* Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes. *Circulation* 2001; 103:1955–1960.
- 39 Holvoet P, Vanhaecke J, Janssens S, *et al.* Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. *Circulation* 1998; 98:1487–1494.
- 40 Holvoet P, Collen D, van de Werf F. Malondialdehyde-modified LDL as a marker of acute coronary syndromes. *JAMA* 1999; 281:1718–1721.
- 41 Hayashida K, Kume N, Murase T, *et al.* Serum soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are elevated in acute coronary syndrome: a novel marker for early diagnosis. *Circulation* 2005; 112:812–818.
- 42 Crisby M, Nordin-Fredriksson G, Shah PK, *et al.* Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases, and cell death in human carotid plaques: Implications for plaque stabilization. *Circulation* 2001; 103:926–933.
- 43 Cominacini L, Anselmi M, Garbin U, *et al.* Enhanced plasma levels of oxidized low-density lipoprotein increase circulating nuclear factor-kappa B activation in patients with unstable angina. *J Am Coll Cardiol* 2005; 46:799–806.
- This study provided a plausible biological link between OxLDL and the inflammation cascade.
- 44 Anselmi M, Garbin U, Agostoni P, *et al.* Plasma levels of oxidized-low-density lipoproteins are higher in patients with unstable angina and correlated with angiographic coronary complex plaques. *Atherosclerosis* 2006; 185:114–120.
- 45 Segev A, Strauss BH, Witztum JL, *et al.* Relationship of a comprehensive panel of plasma oxidized low-density lipoprotein markers to angiographic restenosis in patients undergoing percutaneous coronary intervention for stable angina. *Am Heart J* 2005; 150:1007–1014.
- 46 Naruko T, Ueda M, Ehara S, *et al.* Persistent high levels of plasma oxidized low-density lipoprotein after acute myocardial infarction predict stent restenosis. *Arterioscler Thromb Vasc Biol* 2006; 26:877–883.
- 47 Braun S, Ndrepepa G, von Beckerath N, *et al.* Lack of association between circulating levels of plasma oxidized low-density lipoproteins and clinical outcome after coronary stenting. *Am Heart J* 2005; 150:550–556.
- 48 Diepeveen SH, Verhoeven GW, Van Der PJ, *et al.* Effects of atorvastatin and vitamin E on lipoproteins and oxidative stress in dialysis patients: a randomised-controlled trial. *Journal of Internal Medicine* 2005; 257:438–445.
- 49 Inami S, Okamoto K, Takano M, *et al.* Effects of statins on circulating oxidized low-density lipoprotein in patients with hypercholesterolemia. *Jpn Heart J* 2004; 45:969–975.

- 50 Ndrepepa G, Braun S, von Beckerath N, *et al.* Oxidized low density lipoproteins, statin therapy and severity of coronary artery disease. *Clinica Chimica Acta* 2005; 360:178–186.
- 51 Silaste ML, Rantala M, Alfthan G, *et al.* Changes in dietary fat intake alter plasma levels of oxidized low-density lipoprotein and lipoprotein(a). *Arterioscler Thromb Vasc Biol* 2004; 24:498–503.
- 52 Shimada K, Mokuno H, Matsunaga E, *et al.* Circulating oxidized low-density lipoprotein is an independent predictor for cardiac event in patients with coronary artery disease. *Atherosclerosis* 2004; 174:343–347.
- 53 Meisinger C, Baumert J, Khuseyinova N, *et al.* Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. *Circulation* 2005; 112:651–657.
- This prospective, case–control study showed that elevated OxLDL levels are associated with increased cardiovascular events.
- 54 Steinberg D, Witztum JL. Is the oxidative modification hypothesis relevant to human atherosclerosis?: Do the antioxidant trials conducted to date refute the hypothesis? *Circulation* 2002; 105:2107–2111.