Oxidized LDL - Know what you measure

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Summary

- Oxidized LDL is a widely used marker within oxidative stress and subsequent diseases.
- It is important to define method of detection since several methods for oxidized LDL determinations with divergent results are in use.
- Increased oxidized LDL levels as measured by assays based on the antibody 4E6 are correlated to cardiovascular disease, diabetes, the metabolic syndrome and obesity.

Background

Oxidized low-density lipoprotein (LDL) is widely used as a marker of oxidative stress and subsequent diseases. However, LDL is a complex particle consisting of a lipid core surrounded by apolipoprotein B-100 (Apo B-100).

There are several methods of measuring oxidized LDL, both direct measurement of the oxidized LDL particle and indirect measurements of endogenous antibodies against oxidized LDL. LDL may be altered by oxidation of its core lipids as well as of the surrounding Apo B-100 and there are assays based on antibodies raised against both. The different methods may not generate the same results and it is therefore important to know what you measure.

Measuring oxidized phospholipids

The antibody FOH1a/DLH3 and the antibody E06 are both directed against epitopes in oxidized phospholipids. In the oxidized LDL assay of Tsimikas *et al.* 2003, Plasma levels of oxidized LDL were shown to have a strong correlation with plasma lipoprotein(a) using the E06 antibody (fig. 1). The authors speculate that the oxidized phospholipids may be preferentially transferred to and sequestered by lipoprotein(a) after being released into circulation.

Tsimikas *et al.* found elevated levels of oxidized LDL only in patients with an acute myocardial infarction and were unable to demonstrate any elevated circulating levels of oxidized LDL in patients with stable coronary heart disease (CHD).

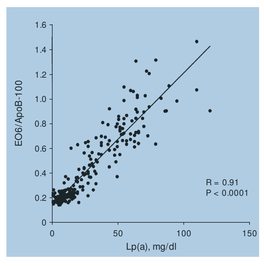

Figure 1.
Correlation between plasma levels of oxidized LDL measured by E06 and Lp(a). Published by Tsimikas *et al.* 2003.

Measuring oLAB

Measuring serum anti-oxidixed LDL antibody (oLAB) levels is sometimes used as an indirect method of determining the degree of LDL oxidation.

Weinbrenner *et al.* (2003) found increased serum oxidized LDL levels to be inversely correlated to serum oLAB levels in stable CAD patients. However, oLAB may be elevated in patients with early-onset peripheral disease (Bergmark 1995), or without significance as a risk factor in CHD patients (Ahmed 1999). The discrepancies among studies of oLAB are difficult to interpret due to differences in techniques and antigens used. Thus, the relevance of oLAB as a marker of LDL oxidation seems unclear.

Measuring oxidized Apo B-100

Mercodia has developed oxidized LDL ELISAs, based on the mouse monoclonal antibody 4E6, developed by the professors Holvoet and Collen at the University of Leuven, Leuven, Belgium. The monoclonal antibody 4E6 is directed against a conformational epitope in the Apo B-100 moiety of LDL that is generated as a consequence of substitution of at least 60 lysine residues of Apo B-100 with aldehydes (Holvoet 2004). This number of substituted lysines corresponds to the minimal number required for scavenger-mediated uptake of oxidized LDL. Substituting aldehydes can be produced by peroxidation of lipids of LDL, resulting in the generation of oxidized LDL. However, lipid peroxidation is not required. Indeed, aldehydes that are released by endothelial cells under oxidative stress or by activated platelets may also induce the oxidative modification of Apo B-100 in the absence of lipid peroxidation of LDL. Thus, the antibody 4E6 is different from the antibodies E06 and FOH1a/DLH3.

The high correlation of oxidized LDL to lipoprotein(a) observed by using the E06 antibody was not observed with the 4E6 antibody (fig. 2). In conclusion, the Mercodia oxidized LDL ELISA is substantially different from the test of Tsimikas *et al.*

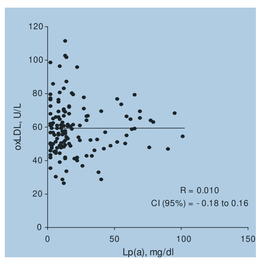

Figure 2.
Correlation between plasma levels of oxidized LDL measured by 4E6 and Lp(a).
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Correlation of oxLDL to LDL-cholesterol

In a study of 148 subjects blood levels of oxLDL (measured with Mercodia oxLDL ELISA) and LDL-cholesterol were determined, and the overall correlation of oxLDL to LDL-cholesterol was found to be about 0.70 (R2=0.4886) (fig. 3). However, if the LDL-cholesterol levels are divided into quartiles the correlation of oxLDL to LDL-cholesterol decreases with increasing blood levels of LDL-cholesterol. (table 1). Thus, the correlation of oxLDL to LDL-cholesterol is lost at elevated LDL-cholesterol levels.

The lack of correlation of elevated LDL levels and oxLDL illustrate that factors like genetic environment and diet may be of more importance for oxLDL levels than the availability of its substrate. It has also been suggested that the size and composition of the LDL particle may affect the oxidation process (Lada 2004).

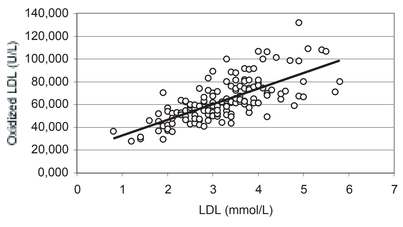

Figure 3.
The overall correlation of oxLDL to LDL-cholesterol measured in 148 seemingly healthy subjects (R^2=0.4886). Dept. of research and development, Mercodia AB.

Quartile	LDL-chol	Subjects	Corr. to oxLDL (R-value)
I	0.8-2.6 mM	1-37 of 148	0.65
II	2.6-3.2 mM	38-74 of 148	0.17
III	3.3-3.8 mM	75-111 of 148	0.13
IV	3.8-5.8 mM	112-148 of 148	0.29
Overall	0.8-5.8 mM	All values (148)	0.70

Table 1. The correlation of oxLDL to LDL-cholesterol measured in 148 seemingly healthy subjects and divided into quartile I, II, III and IV. The overall correlation is 0.70.

Clinical value of Oxidized LDL ELISA determinations

In a study by Johnston *et al.* (2006) oxidized LDL was determined with Mercodia Oxidized LDL ELISA and the results were used in combination with HDL-cholesterol. Johnston and colleagues found the ratio of oxidized LDL to HDL-cholesterol to be a more potent marker in discriminating CAD patients from healthy controls than traditionally used measurements of lipids and lipoproteins as well as Lp-PLA2.

Increased oxidized LDL levels as measured with assays based on the antibody 4E6 have in different studies been correlated to diseases associated with oxidative stress such as cardiovascular disease, diabetes, the metabolic syndrome and obesity (table 2). Monitoring oxidized LDL levels with 4E6 based assays may be of importance in cardiovascular risk assessment in these patients.

Initial work of Prof. Holvoet
Holvoet *et al.* (1998) *Arterioscler Thromb Vasc Biol* 18:100-107
Holvoet *et al*. (1998) *Circulation* 98:1487-1494.

Confirmation of the work of Holvoet
Hulthe, Fagerberg (2002) *Arterioscler Thromb Vasc Biol* 22:1162-1167
Weinbrenner *et al.* (2003) *Atherosclerosis* 168:99-106
Wallenfeldt *et al.* (2004) *J Intern Med* 256:413-420
Meisinger *et al.* (2005) *Circulation* 112:651-657
Anselmi *et al.* (2006) *Atherosclerosis* 185:114-20
Johnston *et al*. (2006) *Int J Cardiol* 113:167-173

Correlation of oxLDL levels with LDL size
Liu *et al.* (2004) *Arterioscler Thromb Vasc Biol* 24:1492-1497
Scheffer *et al.* (2003) *Diabet Med* 20:563-567
Sigurdardottir *et al.* (2002) *J Internal Med* 252: 440-447

OxLDL and the diabetes connection
Kopprasch *et al.* (2002) *Diabetes* 51:3102-3106
Scheffer *et al.* (2003) *Diabet Med* 20:563-567

OxLDL and the metabolic syndrome
Sigurdardottir et al. (2002) *J Intern Med* 252: 440-447
Holvoet *et al.* (2004) *Diabetes* 53:1068-73
Lapointe *et al.* (2007) *Atherosclerosis* 191:362-368

OxLDL and obesity
Couillard *et al.* (2005) *J Clin Endocrinol Metab* 90:6454-6459
Weinbrenner *et al*. (2006) *Am J Clin Nutr* 83:30-35

OxLDL-to-HDL-ch ratio
Johnston *et al.* (2006) *Am J Cardiol* 97:640-645

Impact of statins on oxLDL levels
Holvoet *et al.* (2003) *Arterioscler Thromb Vasc Biol* 23:1444-1448
Ndrepepa *et al.* (2005) *Clin Chim Acta* 360:178-186
Tavridou *et al*. (2006) *Eur J Clin Pharmacol* 62:485-489

Table 2. Publications with the 4E6 antibody.

References

Ahmed *et al.* (1999) *Stroke* 30:2541-6
Bergmark *et al* (1995) *Art Thromb Vasc Biol* 15:441-5
Holvoet *et al.* (2004) *Acta Cardiol.* 59(5): 479-84
Johnston *et al.* (2006) *Am J Cardiol* 97:640-645
Lada *et al.* (2004) *Curr Opin Lipidol.* 15(1):19-24
Tsimikas *et al.* (2003) *J Am Coll Cardio*.l 5;41(3):360-70
Weinbrenner *et al*. (2003) *Atherosclerosis* 168:99-106

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