

## Review Article

# Measurement of Plasma Oxidized Low-Density Lipoprotein and its Clinical Implications

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Oxidized low-density lipoprotein (OxLDL) has been shown to exist in human circulating plasma. Several groups including ours have developed methods for immunologically measuring OxLDL, which have been applied to several clinical, both cross-sectional and prospective, studies. These data clearly show that OxLDL levels correlate well with the severity of cardiovascular diseases. In particular, recent observations suggest that plasma OxLDL levels could be a useful marker for predicting future cardiovascular events; however, substantial differences exist among the different methods of OxLDL measurement. To evaluate the clinical data on circulating OxLDL, a proper understanding of the similarity, differences, and limitation of the methods is needed. This paper summarizes the characteristics of the methods used and recent clinical findings.

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**Key words;** Oxidized low-density lipoprotein, ELISA, Monoclonal antibody, AMI

## Introduction

The appearance of lipid-laden foam cells is a typical feature of atherosclerotic lesions. It is widely accepted that oxidative modification of low-density lipoprotein (LDL) is involved in the development of atherosclerotic lesions through the formation of macrophage-derived foam cells and/or through pro-inflammatory effects on vascular cells<sup>1,2</sup>. Scavenger receptor-mediated uptake of oxidized LDL (OxLDL) induces foam cell formation *in vitro*. Scavenger receptors are a series of receptors that recognize modified LDLs, including OxLDL, that do not bind to native LDL. More than ten different scavenger receptors have been identified and subsequently cloned to date<sup>3-5</sup>. The major contribution of scavenger receptors to foam cell formation and atherogenesis was strongly suggested by studies using macrophages in which genes for scavenger receptors were genetically suppressed<sup>6-9</sup>.

Following the discovery of scavenger receptors, efforts have been undertaken to prove the existence of OxLDL, the physiological ligand(s) of scavenger receptors, *in vivo*. As oxidative modification of LDL is a nonenzymatic posttranslational reaction, genetic techniques cannot be applied to detect OxLDL, but immunological techniques turned out to be very useful. We established a monoclonal antibody (mAb) against OxLDL, DLH3, which binds strongly with OxLDL but not with native LDL, acetylated LDL, or glycated LDL<sup>10</sup>. Since this mAb binds well to oxidized HDL, the binding is not specific for apolipoprotein B (apoB) protein but rather specific for oxidative products of lipids. By isolating antigenic molecules formed in OxLDL, it was found that mAb DLH3 recognized oxidized phosphatidylcholines (PCs), including aldehyde-containing PC (9-CHO-PC)<sup>11</sup>.

Using DLH3 antibody, we established an enzyme-linked immunosorbent assay (ELISA) to measure OxLDL in human circulating plasma<sup>12</sup>. To date, several laboratories, including ours, have succeeded in measuring OxLDL in human plasma using different mAbs<sup>13-16</sup> (Table 1). The ELISA procedures used to determine OxLDL levels in human plasma samples was either a sandwich assay or a competitive assay. With these methods, it was demonstrated that plasma Ox-

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**Table 1.** Comparison of four procedures for OxLDL measurement

Procedure	Itabe's Procedure	Kyowa Medex MX kit	Witztum's Procedure	Holvoet's Procedure	Mercodia kit
ELISA System	Sandwich	Sandwich	Dual-sandwich	Competition	Competition
Ab used	1. DLH3 (anti-OxPC mAb) 2. anti-apoB pAb	1. DLH3 (anti-OxPC mAb) 2. anti-apoB pAb	1. E06 (anti-PhoCho mAb) 2. MB47 3. MB24 (anti-apoB mAb)	1. 4E6 (anti-modified apoB mAb)	1. biotin-4E6 (anti-modified apoB mAb)
Detection	Colorimetric (ALP)	Colorimetric (HRP)	Chemiluminescence (HRP)	Colorimetric (HRP)	Colorimetric (HRP)
Sample prep.	LDL fraction (ultracentrifugation)	Diluted plasma	Diluted plasma	Diluted plasma pre- absorbed with mAb	Diluted plasma
Sample storage	Cannot be frozen	Can be frozen (a reagent for storage is provided by Kyowa)	Cannot be frozen	Can be frozen	Can be frozen
Standard	Cu-OxLDL	Cu-OxLDL	None	MDA-LDL	Pooled patient plasma
OxLDL level in healthy subjects	0.1 ng/ $\mu$ g LDL (ratio)	10 unit/mL plasma (concentration)	0.027-0.42* (ratio of E06 epitope/apoB)	0.7 mg/dL (concentration)	70 units/L (concentration)
Availability	In-house assay	Commercial	In-house assay	In-house assay	Commercial
Advantage	Sensitive; Selective	Takes 1 day	Represents degree of modification	Takes 1 day	Compete in one well
Limitation	Takes 4 days Ultra- centrifuge is needed	Expensive	Chemiluminescence reader is needed	Less selective	Expensive
Reference	12,17,18,20,38,39, 40,42,43,45,47	13,22,28,44,46,49,52	15,16,19,29,35,37,48	14,21,23,27,36, 41,51	27,50,53,54,55

\*The data vary between studies. In some papers, the ratio rather than raw RLU unit was described.

LDL levels increase under certain pathological conditions, including acute myocardial infarction (AMI) and carotid artery atherosclerosis<sup>17-23</sup>. Quantitative analysis of OxLDL in atherosclerotic lesions from the carotid artery shows a massive accumulation of OxLDL in the lesions<sup>18</sup>. These ELISAs for OxLDL measurement are a powerful tool for research on cardiovascular disease; however, slight variations in the procedures followed substantial differences in the results. It should thus be worthwhile to describe the similarity and differences, usefulness, and limitations of these methods for future research on OxLDL.

### Heterogeneity of OxLDL and its Recognition by Specific Monoclonal Antibodies

LDL is a large particle (total molecular mass is about 2,000 kDa) containing one apoB molecule and a large number of triacylglycerol, free cholesterol, cholesteryl ester, and phospholipids molecules. Once LDL

is oxidized, a variety of oxidized lipids are formed, even in the early phase of the reaction, since polyunsaturated fatty acid moieties in the lipids are very susceptible to oxidative reactions. Subsequently, apoB protein is modified by some of the oxidized lipids. In particular, the  $\epsilon$ -amino groups of lysine residues easily generate Schiff's base-adducts with oxidized lipids containing aldehyde groups. Aggregated forms as well as fragments of apoB are produced in OxLDL. OxLDL is thus a mixture of heterogeneously modified lipoprotein particles, and it is important to note that mAbs against OxLDL recognize only parts of modified LDL but not whole particles.

Here, we focus on three anti-OxLDL mAbs, DLH3, E06, and 4E6, which are used to measure OxLDL in circulation. The mAb DLH3 was generated by immunizing mice with a homogenate of human atheroma. DLH3 recognizes OxPC, including 9CHO-PC<sup>11</sup>. E06 also binds to OxPC, but the epitope recognized by this mAb was shown to be phosphorylcho-

line (PhoCho), a hydrophilic moiety of PC. It is interesting that E06 was established from B-cell clones of non-immunized apolipoprotein E-knockout mice, and this antibody was the same as a previously reported T15 antibody that recognizes the PhoCho moiety, on bacterial cell walls<sup>24</sup>. It has also been reported that C-reactive protein binds to OxLDL via the PhoCho moiety just as the E06 mAb binds to OxLDL<sup>25, 26</sup>. 4E6 is a mAb raised by immunizing mice with copper-induced OxLDL. 4E6 binds approximately 1,000 times stronger to OxLDL than native LDL<sup>14</sup>. The epitope of this antibody is a peptide within apoB protein that is exposed to the surface of LDL particles upon the modification of more than 60 lysine residues<sup>27</sup>.

### Comparison of the Two Sandwich ELISA Procedures for OxLDL Measurement, Itabe's procedure and the Kyowa MX Kit

We developed a sandwich ELISA to detect OxLDL in human plasma. The sandwich assay is a type of ELISA in which an antigen is caught between two antibodies<sup>12</sup>. In this procedure, anti-OxPC mAb, DLH3, and an anti-human apoB polyclonal antibody (pAb) are used, such that the antigen detected should be apoB-containing particles that have been modified by OxPC (**Fig. 1A**; this method is referred to as "Itabe's procedure" throughout this article). In this method, the LDL fraction is separated from each human plasma sample prior to the assay. There are two reasons for this step: First, the possibility of detecting oxidized forms of VLDL, IDL, and chylomicron can be eliminated since the LDL fraction ( $d=1.019-1.063$ ) separated by sequential ultracentrifugation was used for the measurement; second, separating the LDL fraction from whole plasma makes the results less scattered and more reproducible. We observed extremely strong coloration in some individual samples when assayed using diluted plasma, but LDL fractions isolated from the same individuals could be applied to ELISA without any problems. It is possible that ELISA can interfere with some materials present in whole plasma. Since the same amounts of LDL fraction ( $5 \mu\text{g}$  protein) are applied to every microtiter well, the data are expressed as "ng OxLDL/ $\mu\text{g}$  LDL protein." The data do not directly represent the OxLDL concentration present in plasma, but rather the ratio of oxidatively modified particles in the LDL fraction.

A limitation of this procedure is that it is not suitable for mass screening; it takes several hours to separate the LDL fraction using ultracentrifugation, and only 8-10 samples can be processed at the same time. Following ultracentrifugation, the resulting LDL

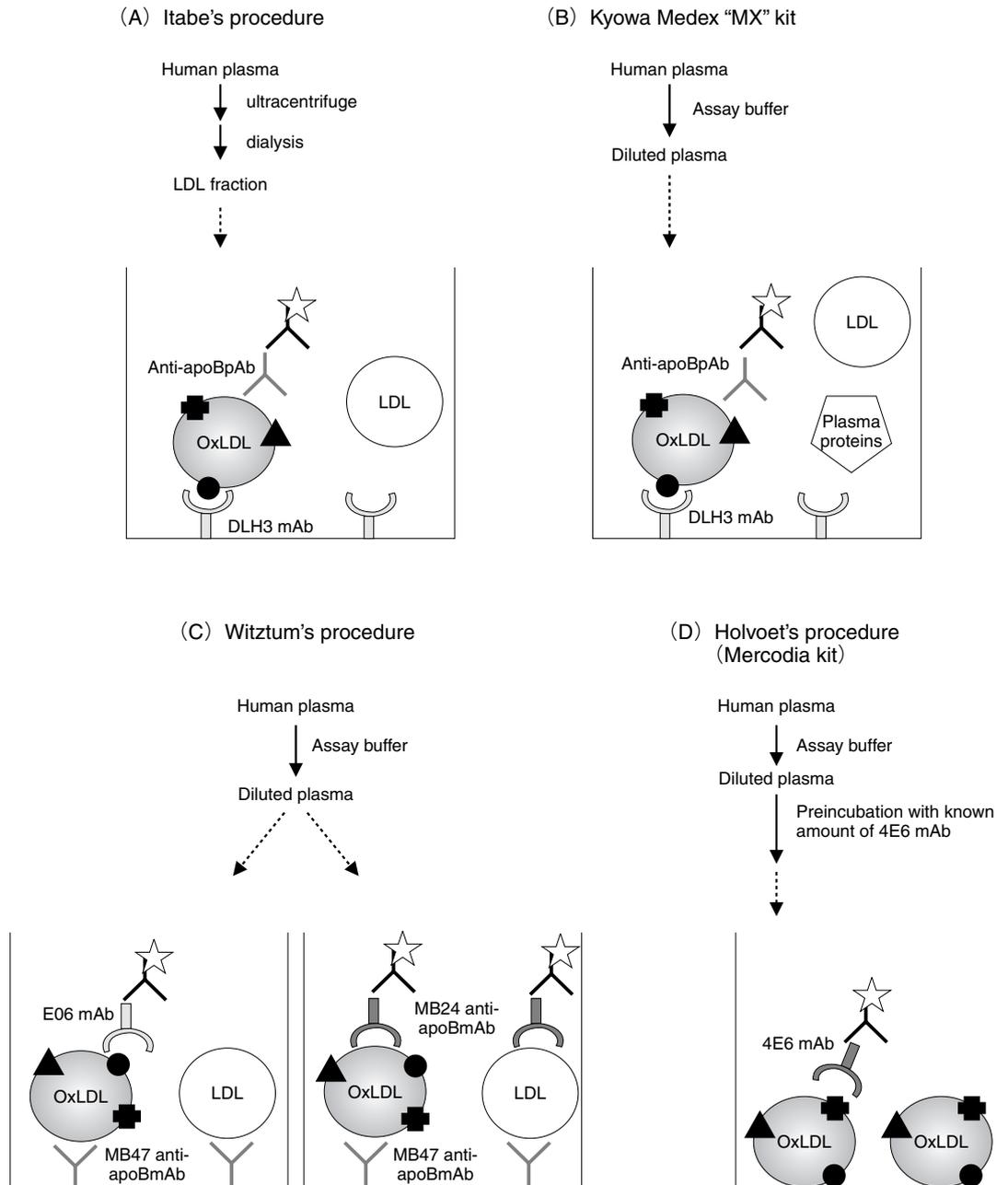
fraction requires dialysis for many hours to remove KBr, and the protein concentration of the final LDL preparation should be measured prior to ELISA. Overall, it takes at least 3 days to run a set of 8-10 samples.

Another sandwich ELISA procedure using DLH3, the same mAb, was developed by Kyowa Medex Inc. (Tokyo, Japan), and they manufactured an ELISA kit for determining OxLDL in plasma<sup>13</sup>. The MX ELISA kit is currently available commercially (in this article it is referred to as the "MX kit"). To make the assay applicable to a larger number of samples, this kit was manufactured to measure OxLDL in diluted plasma (**Fig. 1B**). As it uses a precoated ELISA plate and diluted plasma, the assay can be completed within a day.

Storage of plasma samples is a problem. LDL is prone to aggregate during freezing and thawing, and LDL tends to gradually aggregate even when stored in a refrigerator. Aggregation of LDL particles enhances ELISA coloring not by oxidative modification but by increasing multivalent reactivity against the anti-apoB antibody. Kyowa Medex provides an anti-freeze reagent cocktail for sample storage, which enabled us to store plasma samples at  $-20^\circ\text{C}$  for several months.

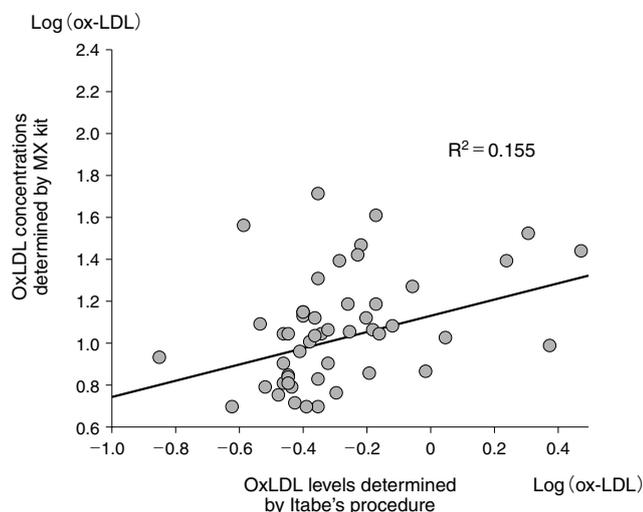
Although these two procedures use DLH3 mAb, the data obtained by the two methods cannot be directly compared. First, Itabe's procedure determines the ratio of oxidative modification in LDL particles, whereas the MX kit provides the concentration of OxLDL in plasma. This difference is a consequence of the sample preparation, either separated LDL or diluted plasma. Actually, the OxLDL concentration determined by the MX kit was shown to correlate with plasma total cholesterol<sup>22, 28</sup>; however, no correlation was observed between plasma total cholesterol and the OxLDL level determined by the sandwich procedure<sup>14, 17</sup>. Second, the OxLDL standards used for the two procedures are prepared under different conditions. The unit of OxLDL used in one assay is not equal to the unit used in the other.

Since these two procedures use the same mAb (DLH3), however, one could postulate that the OxLDL data obtained may show good correlation. In such a case, an OxLDL value for one assay could be converted to a value for another assay by using an appropriate conversion factor. We thus examined the correlation between data obtained from the two assays. During clinical studies of plasma OxLDL levels in 50 patients with AMI, unstable angina pectoris (UAP) and stable angina pectoris (SAP), we measured the OxLDL levels by the two procedures using DLH3 antibody. As shown in **Fig. 2**, there was a moderate correlation between the OxLDL values determined by the two procedures ( $R^2=0.151$ ). Although the two



**Fig. 1.** Four different procedures for OxLDL determination in human circulating plasma. These are all ELISA procedures that use anti-OxLDL mAb; however, several conditions differ as depicted in the figure.

(A) Itabe's procedure. LDL fraction is separated from plasma, and sandwich assay is carried out. (B) Kyowa Medex "MX" kit. The same mAb, DLH3, is used as in (A) and a sandwich assay is conducted, but diluted plasma is applied directly to the assay. (C) Witztum's procedure, in which two ELISAs are run simultaneously, one for OxLDL and one for total apoB. (D) Holvoet's procedure is a competitive assay, in which the amount of 4E6 mAb remaining after preincubation of a known amount of the antibody with diluted plasma was measured. Mercodia's kit is a simple modification of Holvoet's procedure, where 4E6 mAb is incubated with diluted plasma in microtiter wells precoated with OxLDL. Symbols illustrated on OxLDL represent the modified structures formed, some of which are recognized by the anti-OxLDL mAbs used in these assays: ●; oxidized phosphatidylcholines which are recognized by DLH3 and E06, +; a part of apoB protein exposed to the surface after extensive modification of the protein recognized by 4E6, ▲; many other modifications formed during oxidation of LDL such as amino acid residues attached with 4-hydroxynonenal or acrolein. Open star (☆) attached to second antibodies represents enzymes used for detection, i.e. alkaline phosphatase or horseradish peroxidase.



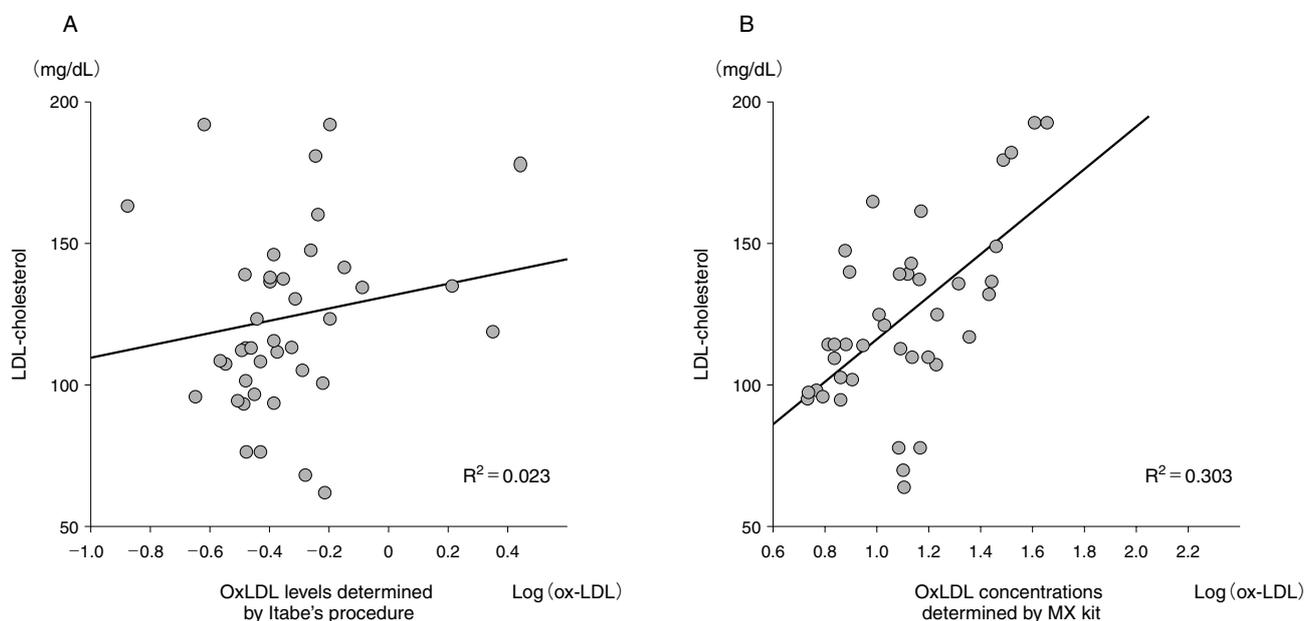
**Fig. 2.** Direct comparison of OxLDL values obtained by Itabe's procedure and the MX kit.

Plasma samples were drawn from patients with AMI (N=20), patients with UAP (N=20), and patients with SAP (N=10). A part of the plasma was used for LDL isolation and subsequent measurement of OxLDL levels using Itabe's procedure. OxLDL concentrations were obtained using an MX kit with the remaining aliquot of the corresponding plasma sample. The values are indicated in logarithmic scale. There were no significant differences among the patient groups in age (AMI,  $66 \pm 10$  years; UAP,  $70 \pm 11$ ; SAP  $67 \pm 12$ ) or plasma total cholesterol concentrations (AMI,  $193 \pm 54$  years; UAP,  $203 \pm 32$ ; SAP  $194 \pm 25$ ).

sets of OxLDL values correlated to some extent, the correlation was very weak to derive an equation to convert an OxLDL value given by one procedure to that given by another procedure. A few samples showed very high OxLDL values when measured using the MX kit but had low OxLDL levels when measured by Itabe's procedure.

There were no statistically significant differences among the AMI, UAP, and SAP groups in plasma total cholesterol (AMI,  $193 \pm 54$  years; UAP,  $203 \pm 32$ ; SAP  $194 \pm 25$ ) and age (AMI,  $66 \pm 10$  years; UAP,  $70 \pm 11$ ; SAP  $67 \pm 12$ ). We also measured other plasma lipid variables, such as triacylglycerol, HDL-cholesterol, and LDL-cholesterol in many of the patients, and did not find any significant differences among the groups. As described above, the OxLDL concentration determined by Itabe's procedure did not correlate with plasma cholesterol<sup>14, 17</sup>. In the current study of cardiovascular patients, we observed that OxLDL values obtained using the MX kit did correlate with LDL-cholesterol concentrations ( $R^2=0.303$ ), as reported in previous studies<sup>22, 28</sup>, whereas little correlation was seen between OxLDL values obtained by Itabe's procedure and LDL-cholesterol concentrations (**Fig. 3**).

The reason for the difference between these two procedures has not yet been clarified; however, we think that separation of the LDL fraction could make



**Fig. 3.** Plasma OxLDL values and LDL-cholesterol.

LDL-cholesterol concentrations of 41 out of 50 patients were measured using the same blood samples as used for OxLDL measurement. Correlation between LDL-cholesterol and OxLDL levels obtained by Itabe's procedure (A) and OxLDL concentrations obtained by the MX kit (B) is shown. The values are indicated in logarithmic scale.

a substantial difference in the measurement of OxLDL. An assay using whole plasma may detect both OxLDL and other oxidized lipoproteins and their complex forms. As LDL is the major apoB-containing lipoprotein in human plasma, the possible contribution of oxidized VLDL and oxidized chylomicron may be very small. Lp(a) is a unique LDL that is covalently modified with a large protein apo(a), which has several cringle repeats and is postulated to be susceptible to oxidation<sup>29, 30</sup>. The plasma concentration of Lp(a) varies greatly among individuals, and the molecular weight of apo(a) also varies among individuals, such that it is difficult to specify the size and density of Lp(a). It is possible that Lp(a) particles are denser than normal LDL, and by separating the LDL fraction from plasma, Lp(a) can be eliminated.

### OxLDL Measurement Procedures Using Other mAbs

As summarized in **Table 1**, two other procedures are also used to determine OxLDL. Witztum and colleagues used the anti-PhoCho mAb E06 and a chemiluminescence detector to develop a dual-sandwich<sup>15, 16</sup> (referred to as “Witztum’s procedure” in this article). In this procedure, two sandwich assays are carried out in parallel with diluted plasma as the sample. OxLDL is determined with anti-apoB mAb MB47 and biotin-labeled E06 mAb in one well; in another well, the total absolute amount of apoB is measured with MB47 and another anti-apoB mAb (MB24) (**Fig. 1C**). Thus, the value obtained is a ratio between oxidized modifications per apoB-containing particle. This value is in part similar to that obtained by Itabe’s procedure where both procedures detect the ratio of oxidized products in LDL; however, the average number obtained by Witztum’s procedure is about 0.3, whereas Itabe’s procedure gave 0.1 ng/ $\mu$ g LDL. The former means that 0.3 mole of oxidation epitope appeared in 1 mole of LDL, and the latter means that oxidation is found in 1 particle in 10,000 LDL particles. Witztum’s value includes the degree of modification; thus, if 10 PhoCho moieties appear on an LDL particle, it will be counted as 10. Itabe’s value would count moderately oxidized and heavily oxidized LDL particles similarly<sup>31</sup>. This apparent discrepancy may suggest that a limited number of LDL particles undergo oxidation to generate OxLDL particles with multiple modification epitopes *in vivo*.

Holvoet *et al.* carried out a simple competitive ELISA to measure OxLDL concentrations in diluted plasma<sup>14</sup> (we refer to this as “Holvoet’s procedure”). In this article, a sample of diluted plasma is preincubated

with a known amount of 4E6 mAb and is then transferred to a well coated with OxLDL (**Fig. 1D**). The more OxLDL present in plasma, the more 4E6 mAb is consumed in preincubation. Apparently, the value obtained by this procedure is a concentration of OxLDL in plasma. Since only one mAb is used, the possibility that the antigen detected by this assay, which is a conformationally changed apoB, may be formed by any stress in addition to oxidation will be investigated in a future study. An OxLDL-measuring kit based on Holvoet’s procedure is commercially available from Mercodia Inc. (Sweden). There are several minor differences between the original procedure of Holvoet’s and the Mercodia kit, but it is reported that the two measures of the same plasma sample correlate fairly well (correlation efficient  $R^2=0.63$ )<sup>27</sup>. Diluted plasma is used in both procedures, and similar processing of the plasma sample may be the major reason for this correlation.

### *In vivo* OxLDL Measurement and *ex vivo* Oxidation Assay

In earlier studies, *ex vivo* assay of LDL oxidation (the lag time assay), originally introduced by Dr. Esterbauer<sup>32</sup>, was extensively used to estimate the susceptibility of LDL to oxidative stress. LDL can be easily oxidized when incubated with micromolar concentrations of  $\text{Cu}^{2+}$ . When the oxidation reaction is monitored by UV absorbance at 233 nm for conjugated double bonds, the reaction proceeds quickly after a certain lag time (approximately 90–120 min). It is predicted that a short lag time indicates the increased OxLDL levels in plasma LDL, because the more lipid peroxides that are present in LDL, the more susceptible LDL is to  $\text{Cu}^{2+}$ -induced oxidation. It is noteworthy that this assay does not detect the OxLDL already present in plasma. Despite the short lag time, in this assay, the sample was not oxidized unless it was incubated with copper ion. The lag time of the LDL oxidation assay is largely affected by the content of lipophilic antioxidants, such as  $\alpha$ -tocopherol, in LDL particles<sup>33</sup>.

Administration of  $\alpha$ -tocopherol does not seem to affect the circulating levels of OxLDL in patients receiving hemodialysis (unpublished data). The reason for this observation is not yet clear, but two points should be considered. First, circulating LDL may contain a sufficient amount of antioxidants to protect LDL particles from oxidative modification under *in vivo* conditions. Second, the way LDL is oxidized *in vivo* may differ from  $\text{Cu}^{2+}$ -induced oxidation *in vitro*. Moreover,  $\alpha$ -tocopherol could act as a pro-oxidant rather than an anti-oxidant through a tocopherol-me-

diated propagation (TMP) mechanism *in vivo*<sup>34</sup>). This issue will be solved when the precise mechanism by which LDL is oxidized *in vivo* is clarified.

### Clinical Studies Using the ELISA Determination of OxLDL

Clinical observations of the involvement of circulating OxLDL in cardiovascular diseases are accumulating with the use of these ELISA procedures. As discussed above, the raw values given by these procedures cannot be compared, but there are intriguing correspondences in the behavior of OxLDL in certain pathological conditions. Circulating OxLDL levels were elevated in patients with coronary artery disease<sup>17, 19-22, 35-37</sup>, in patients with carotid arteriosclerosis<sup>18, 38-40</sup>, in patients with transplant-associated atherosclerosis<sup>16, 23, 41</sup> and in patients receiving hemodialysis<sup>12, 14, 42</sup>. OxLDL measures were also significantly elevated in patients with diabetes mellitus<sup>43, 44</sup>. A positive correlation was reported between OxLDL levels and endothelial function or vasodilation response<sup>15, 42, 45, 46</sup>. From these observations, it is now believed that circulating OxLDL levels in human plasma are significantly affected in some diseased conditions. OxLDL is a mixture of heterogeneously modified particles as described above. It is possible that the four procedures for OxLDL measurement may detect slightly different repertoires of OxLDL particles. Therefore, it can be considered that these OxLDL measures may not determine the absolute amount of OxLDL but can indicate the relative changes in OxLDL levels *in vivo*.

Plasma OxLDL levels may change in individuals during different stages of a disease. Several reports have shown that OxLDL levels increase in the acute phase immediately after AMI, cerebral infarction, or percutaneous transluminal coronary angiography (PTCA) intervention<sup>17, 37-39, 47, 48</sup>. When OxLDL levels were followed in patients during hospitalization and after discharge, the highest OxLDL levels were observed in acute phases, 1 to 3 days after the event, after which the OxLDL levels gradually declined to near normal levels. One possible explanation for the temporal increase in circulating OxLDL during the acute phase of an AMI or stroke is that OxLDL can be released from ruptured plaques into the circulation at the time of occurrence of infarction.

A few studies have reported the effects in patients treated with statins. Inami *et al.* measured OxLDL concentrations in hypercholesterolemic patients, using the MX kit, before and after 12 weeks of statin treatment<sup>49</sup>. Administration of pravastatin reduced LDL-cholesterol by 22% and OxLDL concentration by

21%; thus, the ratio of OxLDL to LDL did not seem to change much. On the other hand, fluvastatin treatment reduced the OxLDL concentration by 51%, while LDL-cholesterol decreased by only 15%. Fluvastatin is known to possess an anti-oxidant property *in vitro* along with inhibitory action for HMG-CoA reductase, and the above observation suggests that fluvastatin may have an inhibitory effect on the oxidation of LDL *in vivo*.

The effect of atorvastatin on OxLDL levels was examined in the MIRACLE trial<sup>35</sup>. In this study, 2341 patients with AMI or UAP were treated with either atorvastatin or placebo for 16 weeks. Plasma samples were taken within 4 days from the beginning of the test and at the end of the 16-week treatment, and the OxLDL/apoB ratio was determined by Witztum's procedure. Treatment with atorvastatin reduced apoB concentrations by 34%; however, the OxLDL/apoB ratio increased by 9.5%. A strong explanation for the apparently unpredicted results was not given, but the authors raised the possibility that OxPC molecules may be condensed in some LDL particles. It is interesting to speculate that the metabolic rate, or clearance efficiency, of OxLDL and native LDL may be differentially affected by statin treatment.

OxLDL has also been measured in clinically healthy individuals. In the AIR study, Hulthe and Fagerberg reported that OxLDL concentrations determined by Mercodia's kit correlated with intima-media thickness and plaque occurrence, which suggests that OxLDL may be closely coupled with the development of atherosclerotic lesions<sup>50</sup>. The Health ABC study tested 3,033 persons in a well-functioning older population and found that OxLDL concentrations (Holvoet's procedure) in subjects with metabolic syndrome ( $1.45 \pm 0.82$  mg/dL) were significantly higher than in those without metabolic syndrome ( $1.23 \pm 0.67$  mg/dL) ( $P < 0.0001$ )<sup>51</sup>.

### Prospective Possibilities of Circulating OxLDL Measures

Recently, several studies proposed the possibility of using OxLDL measurement in the prediction of future cardiovascular events. Naruko *et al.*, reported OxLDL levels in patients with AMI during their stay in the hospital and after cardiovascular accident by utilizing Itabe's procedure<sup>47</sup>. The AMI patients ( $n = 102$ ) had an OxLDL value of  $1.55 \pm 1.21$  ng/5  $\mu$ g LDL at admittance to the hospital, which was 3-times higher than the value in control subjects, and the OxLDL value decreased to  $0.71 \pm 0.47$  ng/5  $\mu$ g LDL at discharge. Twenty-five of the 102 AMI patients experienced re-

stenosis within 6 months. The OxLDL level of the 25 restenosis patients was  $1.03 \pm 0.65$  ng/5  $\mu$ g LDL, which was significantly higher than in patients without restenosis ( $0.61 \pm 0.34$  ng/5  $\mu$ g LDL). It is interesting to speculate that high OxLDL levels during the stable phase suggest that the patients were being exposed to strong oxidative stress or other unstable plaques.

For one year Shimada *et al.* followed 238 patients with more than 50% stenosis angiographically<sup>52</sup>. OxLDL concentrations were measured using the MX kit before the angiogram examination. The OxLDL concentration of 76 patients, who had cardiovascular events within 1 year, was significantly higher when compared with other patients. In the highest quartile of OxLDL, 28 of 59 patients experienced events within 1 year in contrast to the 13 patients in the lowest quartile.

The MONICA/CORA study was conducted to follow 9796 subjects for up to 6 years<sup>53</sup>. Plasma OxLDL concentrations were measured by Mercodia kit. Acute myocardial diseases occurred in 88 men during the 6-year follow-up, and these men were compared with 258 age-matched control men. The patient group had significantly higher OxLDL and CRP concentrations than the control group. The OxLDL and CRP concentrations did not correlate with each other, suggesting that these indexes are independent risk markers. The results of these studies suggest that OxLDL measurement may be a useful predictive marker for cardiovascular diseases.

### Some Controversial Studies

Using Itabe's procedure, we showed previously that plasma OxLDL levels in patients with AMI are significantly higher than those in patients with either UAP or SAP or in controls<sup>17, 20</sup>. Tsimikas *et al.* reported that temporal increases in plasma OxLDL levels (Witztum's procedure) strongly reflect the presence of acute coronary syndromes<sup>19</sup>. However, in contrast with these data, Braun *et al.* recently investigated circulating concentrations of plasma OxLDL in 386 patients with SAP, 109 patients with UAP, and 192 patients with AMI, using the Mercodia kit and reported no significant association with coronary artery disease<sup>54</sup>. When the half with higher OxLDL concentrations were compared with the half with lower concentrations, no significant difference was detected in the event rate, which included restenosis and AMI, during the 10-year follow-up (27.2% versus 25.4%). It is noteworthy that the different procedures for OxLDL measurement may detect different types of OxLDL, and in such a case, the clinical and diagnostic signifi-

cance of the OxLDL level could vary. Future studies should take this issue into consideration.

Metabolic syndrome is a major cause of cardiovascular diseases. Sjogren *et al.* examined OxLDL concentrations (Mercodia kit) and other metabolic parameters in 289 healthy men (age 62-64)<sup>55</sup>. Twenty-two of 289 subjects appeared to have metabolic syndrome. The mean OxLDL concentration in 22 metabolic syndrome subjects was slightly higher than in others; however, the difference was not significant. The clinical significance of OxLDL measures is being further studied. The usefulness and limitations of OxLDL measurement should be carefully studied in future research.

A number of reports raised other plasma components as possible markers for cardiovascular diseases to date. A couple such as C-reactive protein (CRP), soluble forms of lectin-type OxLDL receptor-1, or small dense LDL are of interest, because CRP and sLOX-1 could bind to OxLDL and small dense LDL is thought to be susceptible to oxidative modification<sup>56-58</sup>. It is certainly necessary to correlate plasma OxLDL levels and these other markers and their clinical relevance in the future.

### Conclusion

It has been more than 10 years since OxLDL was first measured in human plasma. Since then, the determination of human circulating OxLDL has become popular with the introduction of several ELISA procedures, partly because a strong correlation has been found between circulating OxLDL measures and cardiovascular disease, and also partly because of the commercial availability of kits for OxLDL measurement. Accumulating data support the importance of OxLDL as a useful diagnostic marker and possibly as a prognostic tool. To fully understand the biological and clinical significance of OxLDL measures, it is useful to understand the differences and limitations of the different ELISA procedures used for OxLDL determination.

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