presurgery evaluation showed that this patient had a calcium concentration within the reference interval. With these findings the diagnosis of PHPT might have been missed in a patient in whom the diagnosis was not yet established. The T-iPTH and iPTH concentrations were within the manufacturers’ reference intervals.

The present report confirms that third-generation PTH assays do not measure only PTH(1–84). In our study, although close to 5% of PTH concentrations were higher when measured with third- than with second-generation assays, this did not influence the diagnosis of PHPT in the large series of consecutive patients with surgically and histologically confirmed PHPT without any feature of malignancy and with markedly lower PTH concentrations than those reported previously. Further studies are needed to evaluate whether the presence of this unexpected PTH profile is predictive of malignancy.

References


Background: Oxidized LDL (ox-LDL) plays an important role in the pathogenesis of coronary heart disease (CHD). Several tests for circulating ox-LDL have been published. We believe it is critical to carefully evaluate these assays because small differences in performance may have profound effects when results are compared; we therefore compared the analytical and clinical performances of 2 assays: one developed in our laboratory and a commercial assay (Mercodia) that uses the same monoclonal antibody (4E6).

Methods: We determined the variance of ox-LDL in both tests, including its longitudinal stability (n = 225; 3 time points per person) and its diagnostic accuracy, by ROC analysis of 95 consecutive CHD patients and 20 controls.

Results: The between-person variability was 77% for the in-house assay (with the remaining 23% being within-person and analytical variance) and 74% for the commercial assay. For comparison, previously reported values were 66% for high-sensitivity C-reactive protein and 82% for total cholesterol. The areas under the curves for CHD in the 2 assays were identical (0.85). The odds ratios (logistic regression) for CHD among persons with high ox-LDL (≥15 mg/L) compared with persons with low ox-LDL were not different: 4.3 (95% confidence interval, 1.4–12) for the in-house assay and 3.3 (1.1–10) for the commercial assay.

Conclusions: The longitudinal stability of ox-LDL, as assessed by multiple measures in people over time, is similar to that of total cholesterol and high-sensitivity C-reactive protein. Both assays tested similarly distinguish between healthy controls and CHD patients.

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Oxidized LDL (ox-LDL) plays an important role in the pathogenesis of atherosclerosis (1–3). We and others have demonstrated that subclinical atherosclerosis (4) and clinical coronary heart disease (CHD) (5–9) are associated with higher concentrations of circulating ox-LDL. Recently, we showed, in older adults in the Health, Aging, and Body Composition (Health ABC) cohort (10), that high CHD risk status (based on Framingham score) before
CHD events is associated with high concentrations of circulating ox-LDL (7, 10) and that increased ox-LDL predicts future myocardial infarction (11). The latter has been confirmed in middle-aged adults in the MONICA/KORA Augsburg cohort (12) and the FRISC-II (Fragmin and Fast Revascularization in Instability in Coronary Artery Disease Trial) cohort (13).

For measurement of circulating ox-LDL, we have developed and used an in-house monoclonal antibody (mAb 4E6)-based competitive ELISA (14). This antibody is directed against a conformational epitope in the apolipoprotein B-100 (apoB-100) moiety of LDL that is generated as a consequence of substitution of at least 60 lysine residues of apoB-100 with aldehydes. This number of substituted lysines corresponds to the minimal number required for scavenger-mediated uptake of ox-LDL. The substituting aldehydes can be produced by peroxidation of lipids of LDL, leading to the generation of ox-LDL. Aldehydes that are released by endothelial cells under oxidative stress or by activated platelets may also induce oxidative modification of apoB-100 in the absence of peroxidation of lipids of LDL. Recently, Mercodia (Uppsala, Sweden) began marketing a competitive ELISA for ox-LDL (product no. 10-1158-01) that uses the same assay technology and the same specific mouse mAb, 4E6.

We believe that it is critical to carefully evaluate assays used in population and clinical research because small differences in performance (such as sensitivity or specificity) may have profound effects when results are analyzed. The aim of the present study, therefore, was to compare the analytical and clinical performance of the Leuven in-house test (test 1) with that of the Mercodia assay (test 2).

In the Leuven test, a plasma sample was incubated with mAb-4E6 and then applied to the wells of a microtiter plate, where a competitive binding reaction was set up between ox-LDL in the plasma sample and ox-LDL immobilized in the microtiter plate well. After a washing step, the antibody bound to the immobilized ox-LDL was quantified with a horseradish peroxidase–conjugated antibody and with the peroxidase substrate 1,2-benzene diamine (14). The Mercodia Oxidized LDL Competitive ELISA uses a similar technology, in which ox-LDL in the sample competes with ox-LDL bound to the microtiter well for the binding of mAb-4E6; however, the antibody is biotin-labeled. After a washing step, the biotin-labeled antibody bound to the well is detected with horseradish peroxidase–conjugated streptavidin and the peroxidase substrate 3,3′,5,5′-tetramethylbenzidine.

We determined the variance of ox-LDL in both tests, using 675 samples: 3 samples obtained from 225 healthy volunteers [mean (SD) age, 34 (10) years] at 3 different time points, 6 weeks to 9 months apart. Between August 1987 and May 1988, 5-mL blood samples were collected at the Laboratory for Clinical Biochemistry Research at the University of Vermont from donors (with appropriate informed consent) as part of a process in which each donor had samples collected at multiple time points. Because this blood was collected for a variety of purposes, the anticoagulant was citrate-phosphate-dextrose-adenine erythrocyte preservative solution from Baxter-Senwall. Samples were processed within 3 h (storage at 4 °C until processing), which included centrifugation at 100,000 g/min to be certain the plasma was platelet free. Samples were aliquoted and stored at −80 °C. Samples selected for the biovariability study had not been thawed since the time of blood collection and had 3 time points available for each of the 75 participants. Samples were coded and sent to Leuven, where ox-LDL concentrations were measured; data were then sent to Vermont for analysis by STATA (Stata Corp) statistical software running the LONEWAY command. We have interpreted the intraclass correlation coefficient as being the percentage variation attributable to between-person variation (15). The between-person variability was 77% for test 1 (with the remaining 23% being within-person and analytical variance) and 74% for test 2. For comparison, previously reported values for other known cardiovascular risk factors were 66% for high-sensitivity C-reactive protein and 82% for total cholesterol (15). We then compared the reactivity of the respective calibrators. The calibrator of the Leuven in-house assay was malondialdehyde (MDA)-modified LDL, which typically contained 60 to 90 substituted lysine residues per apoB-100 molecule. We used MDA-modified LDL as a proxy for ox-LDL because its reactivity was identical, although the former was more stable. The epitope that depended on protein modification independent of the degree of lipid oxidation was equally exposed in MDA-modified LDL and ox-LDL that contained the same number of substituted lysines. The calibrators that come with the Mercodia assay are prepared from pooled patient plasma samples, which after lyophilization are calibrated against the Mercodia Oxidized LDL Reference Standard. Concentrations of ox-LDL in plasma samples are expressed in arbitrary units/L. As shown in Fig. 1, we found a close relationship between the reactivity of the Leuven and Mercodia calibrators in test 1. Similar data were obtained when both calibrators were analyzed in test 2.

We also compared the analytical and clinical performance of both tests. For this comparison, after receiving institutional approval and informed consent we enrolled 95 consecutive patients (May 2004), exclusively nonstatin users who were scheduled for angiography, at the Cardiology Unit of the University Hospital, Leuven (Table 1). We collected blood samples between 0800 and 0900 in the morning, after an overnight fast. Immediately after withdrawal, blood samples (4–5 mL) were placed in ice-chilled tubes containing EDTA (Vacutainer; Becton Dickinson Ref. 36886). Plasma samples were rapidly separated by centrifugation at 100 000 g per min at 4 °C, and then divided into 500-μL aliquots, which were then frozen and stored at −80 °C. We also analyzed samples from 20 healthy controls (staff members), as described previously (6).

As shown in Fig. 1B, we found a close relationship between the reactivity of the patient plasma samples in both assays. As indicated by the data in panels C and D of Clinical Chemistry 52, No. 4, 2006 761
Fig. 1, variations in LDL-cholesterol explained 26% and 39% of the variation in ox-LDL measured in tests 1 and 2, respectively.

Retrospectively, 51 patients were found to have coronary artery disease: 17 presented with angiographically confirmed 1-vessel disease (≥50% stenosis), 12 with 2-vessel disease, and 4 with 3-vessel disease. Another 8 patients had previous acute coronary syndrome with <50% stenosis. Fourteen non-CHD patients had cardiovascular risk equivalents: noncoronary forms of clinical atherosclerotic disease, diabetes, and/or a 10-year risk for CHD events >20% by Framingham scoring (16). They were considered high-risk patients. Thirty patients were low-risk patients.

LDL-cholesterol concentrations among CHD and non-CHD patients were similar (Table 1). CHD patients had somewhat lower HDL-cholesterol and higher triglyceride and ox-LDL concentrations.

We also tested the diagnostic accuracy of ox-LDL by ROC curve analysis. We first compared CHD with non-CHD patients, not taking into account the risk profiles of the non-CHD patients. The areas under the curves for the 2 tests were identical: 0.72 (Table 1). The positive predictive value of test 1 was 73%, and the negative predictive value was 65%. For test 2, the positive and negative predictive values were both 70%. We next compared CHD patients with healthy controls. The areas under the curves were identical: 0.85 (Table 1). The positive predictive value of test 1 was 93%, and the negative predictive value was 62%. For test 2, the positive and negative predictive values were 90% and 69%, respectively. The odds ratios (logistic regression) in this small study for CHD among persons with high ox-LDL (≥15 mg/L), compared with persons with low ox-LDL were not different: 4.3 (95% confidence interval, 1.4–12) for test 1, and 3.3 (1.1–10) for test 2.

The main goal of this study was to evaluate the analytical performance and clinical applicability of 2 assays for ox-LDL: one in-house assay and one commercial assay. We did this because we recognize the need to critically evaluate the preanalytical and analytical variance for assays reported in the literature. The longitudinal stability of ox-LDL concentrations, as assessed by multiple measures in people over time, is similar to that of total cholesterol and high-sensitivity C-reactive protein. To be able to compare data obtained with the commercial assay and the in-house assay, we calculated a conversion factor. We found that 15 mg/L in the in-house assay (this was the mean value of persons at high cardiovascular risk but without any diagnosis of CHD) corresponded with 50 units/L in the commercial assay.

The main finding of our study was that both assays had similar precision for distinguishing between healthy individuals and patients with CHD. Both assays similarly differentiated between the groups of healthy individuals and patients with CHD and, to a lesser extent, between
CHD and non-CHD patients, particularly when the individuals had a high calculated risk. However, we note that the results of this study cannot be extrapolated to other clinical settings. Diagnostic accuracy depends strongly on patient selection and the type of multidrug treatment at the time of blood collection and should be evaluated in large-scale trials. A limitation of both assays is that they are not yet automated. Our data suggest, however, that both assays are similar in analytical performance and clinical applicability and that both assays warrant further investigation of the clinical utility of ox-LDL screening as a novel tool for vascular risk prediction. This hypothesis is supported by recent studies showing that ox-LDL predicted risk for future events in the Health ABC, the MONICA-KORA, and the FRISC-II cohorts (11–13). Our data also show that ox-LDL can be measured in samples that have been stored for more than 15 years if they had been collected carefully and had not been thawed.

This study was supported in part by the Fonds voor Wetenschappelijk Onderzoek–Vlaanderen (Program G027604) and by the Interuniversity Attraction Poles Program–Belgian Science Policy (P5/02). We thank Hilde Bernar and Laura De Velder for excellent assistance. P. Holvoet is an inventor of the assay for oxidized LDL, for which patents have been granted in Europe (EP 1110092) and the United States (US Patents 6,309,888 and 6,727,102). All rights on the invention are assigned to KU Leuven Research & Development, VZW, the technology transfer unit of the Katholieke Universiteit Leuven, which also receives any royalties. The study described in this Table 1. Analytical performance of tests for ox-LDL, characteristics of study cohort, and clinical applicability of ox-LDL.

<table>
<thead>
<tr>
<th>Characteristics of patients</th>
<th>Ox-LDL test 1 (n = 675)</th>
<th>Ox-LDL test 2 (n = 675)</th>
<th>Variance</th>
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</thead>
<tbody>
<tr>
<td>Analytical (within-person) variance, %</td>
<td>23 (16)</td>
<td>26 (9.7)</td>
<td></td>
</tr>
<tr>
<td>Between person variance, %</td>
<td>77</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Estimated reliability of a personal mean (n = 9)</td>
<td>0.97</td>
<td>0.96</td>
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</table>

<table>
<thead>
<tr>
<th>ROCS analysis</th>
<th>Ox-LDL test 1</th>
<th>Ox-LDL test 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUCb</td>
<td>Sensitivity, %</td>
<td>Specificity, %</td>
</tr>
<tr>
<td>CHD vs non-CHD</td>
<td>0.72 (0.62–0.81)</td>
<td>67</td>
</tr>
<tr>
<td>CHD vs healthy controls</td>
<td>0.72 (0.61–0.80)</td>
<td>78</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prevalence of CHD according to ox-LDL</th>
<th>Low ox-LDL</th>
<th>High ox-LDL</th>
<th>P for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ox-LDL test 1</td>
<td>4.3 (1.4–12)c</td>
<td>3.3 (1.1–10)c</td>
<td>NS</td>
</tr>
<tr>
<td>Ox-LDL test 2</td>
<td>4.3 (1.4–12)c</td>
<td>3.3 (1.1–10)c</td>
<td>NS</td>
</tr>
</tbody>
</table>

- a NS, not significant; ACS, acute coronary syndrome; CVD, cardiovascular disease; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; SBP, systolic blood pressure; AUC, area under the curve.
- b Mean (95% confidence interval).
- c Odds ratio and 95% confidence intervals in fourth quartile compared with first quartile. Cutoff value for ox-LDL 1 was 15 mg/L in test 1 and 65 units/L in test 2.
paper was not supported by Mercodia, and none of the participants were acting as consultants for Mercodia.

References


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