Reactivity of Direct Assays for Low-Density Lipoprotein (LDL) Cholesterol Toward Charge-Modified LDL in Hypercholesterolemia

Bo Zhang, PhD; Emi Kawachi, BSc; Akira Matsunaga, MD, PhD; Satoshi Imaizumi, MD, PhD; Keita Noda, MD, PhD; Yoshinari Uehara, MD, PhD; Shin-ichiro Miura, MD, PhD; Kazuhiko Yoshinaga, PhD; Masahide Kuroki, MD, PhD; Keijiro Saku, MD, PhD

Background: The aim of the present study was to compare 2 direct measurements for low-density lipoprotein cholesterol (LDL-C) with the Friedewald calculation (LDL-C [F]) in serum and their relationship with size- and charge-based LDL subfractions in serum ultracentrifugation fractions in patients with hypercholesterolemia (HC).

Methods and Results: Serum samples from 283 HC patients who participated in a statin trial (the PATROL trial) were analyzed. Homogeneous assays for LDL-C were performed using reagents from Sekisui Medical (LDL-C [Se]) and Kyowa Medex (LDL-C [Ky]). Charge-based LDL subfractions were analyzed by capillary isotachophoresis (cITP). In whole serum in HC patients at baseline, LDL-C (Se) and LDL-C (Ky) negatively and positively deviated, respectively, from LDL-C (F). The negative deviation of LDL-C (Se) from LDL-C (F) increased with increasing LDL-C, while the positive deviation of LDL-C (Ky) from LDL-C (F) was positively correlated with charge-modified LDL (cmlDL) as analyzed by cITP. In serum d>1.006 g/ml and >1.040 g/ml fractions (LDL and small, dense LDL fractions, respectively), the deviation of LDL-C (Ky) from LDL-C (Se) was positively correlated with LDL-apoB (the number of LDL particles) and cmlDL.

Conclusions: The 2 homogenous assays for LDL-C differed with regard to reactivity toward LDL particles and cmlDL in patients with HC. Direct measurement of LDL-C that reflects modified LDL, could be a better marker for the risk of coronary heart disease. (Circ J 2012; 76: 2241–2248)

Key Words: Capillary isotachophoresis; Charge-modified LDL subfraction; Electronegative LDL; Homogenous assay for LDL-C; Hypercholesterolemia

Elevated low-density lipoprotein cholesterol (LDL-C) is an established major risk factor for coronary heart disease (CHD), and target values of LDL-C have been recommended for the diagnosis and treatment of hypercholesterolemia (HC). The calculation of LDL-C (LDL-C [F]) from the total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels using the Friedewald formula (TC–HDL-C–TG/5) is a common approach for determining LDL-C level in the fasting state. Direct methods for measuring LDL-C using homogeneous assays with an autoanalyzer overcome the limitations of the Friedewald calculation, which cannot be used for samples containing chylomicrons (non-fasting samples), samples from patients with type III hyperlipoproteinemia, or samples with TG >400 mg/dl.

Good agreements between homogeneous assays for LDL-C developed by different reagent manufacturers and LDL-C (F) have been demonstrated in large population studies, including the Framingham Offspring Study, the Iwate-Kenpoku cohort study, and the Women’s Health study, in which reagents from Kyowa Medex, Sekisui Medical and Roche Diagnostics, respectively, were used for the direct measurement of LDL-C.

The reactivity of homogeneous assays for LDL-C toward abnormal lipoproteins, however, has also raised some concerns. Among 4 homogeneous assays for LDL-C from different reagent manufacturers, only the Kyowa Medex LDL-C assay was strongly correlated with the results of the beta-quantification.
Ultracentrifugation was performed for 2.5 h at 10°C in a TLA 120.2 rotor. Various forms of modified LDL including small, dense LDL (sdLDL), oxidized LDL, and glycated LDL exist in human plasma. Both oxidation and glycation of LDL increase the negative electric charge of LDL. Therefore, charge-modified LDL (cLDL) is an integrated measure of modified LDL, and is analyzed as electronegative LDL by anion-exchange chromatography and as fast-migrating LDL (fLDL) by capillary isotachophoresis (cITP). Hypertriglyceridemia is associated with both increased sdLDL and electronegative LDL, which are atherogenic. The reactivity of homogeneous assays for LDL-C toward atherogenic modified LDL has not been examined, which may, however, be clinically important.

The present study compared the 2 most widely used homogeneous assays for LDL-C in clinical laboratories in Japan with regard to their relationship to the Friedewald formula in serum, and their Reactivity toward size- and charge-based LDL subfractions in serum ultracentrifugation fractions in patients with hypercholesterolemia before and after statin treatment.

Methods

Patients

The subjects consisted of 283 HC patients with CHD or risk factors for CHD who participated in the PATROL trial, in which HC patients were treated with 3 strong statins at low dose (atorvastatin 10 mg, rosuvastatin 2.5 mg, and pitavastatin 2 mg) for 4 months. Two patients who had baseline TG ≥400 mg/dl were excluded. This study protocol was approved by the Independent Review Board (IRB) of Fukuoka University Hospital. Overnight fasting blood samples were drawn from each patient. Serum samples were kept at 4°C after separation and were measured with regard to serum TG, HDL-C, and LDL-C and other clinical laboratory data on the same day that blood was drawn without further storage in a central clinical laboratory (SRL, Fukuoka, Japan). After the measurement, serum samples were immediately frozen and stored at −80°C for comparison of the LDL-C direct measurement methods and the analysis of lipoprotein subfractions in serum and serum ultracentrifugation fractions at baseline and at the end of the study.

Laboratory Measurements

Serum levels of TC, TG and HDL-C were measured by enzymatic methods using reagents from Sekisui Medical (Tokyo, Japan), and levels of apolipoprotein (apo) A1, B, and E were measured using turbidimetric immunoassay methods, as described previously. Serum remnant-like particle cholesterol (RLP-C) levels were measured using a Metaboread RemL-C reagent kit for an autoanalyzer, as described previously.

EDTA was added to serum samples at a final concentration of 2 mmol/L to prevent the possible modification of LDL particles during separation and analysis. Serum ≥1.006 g/ml and >1.040 g/ml fractions were separated using quantitative ultracentrifugation as described previously. Ultracentrifugation was performed for 2.5 h at 10°C in a TLA 120.2 rotor in an Optima MAX-XP Benchtop Ultracentrifuge (Beckman Coulter, Fullerton, CA, USA). In the serum ≥1.006 g/ml fraction, TG-rich lipoprotein (TRL) including very-low-density lipoprotein (VLDL) was depleted, and classically defined LDL particles, ie, intermediate-density lipoprotein (IDL) (d=1.006–1.019 g/ml) and the bulk of LDL (d=1.019–1.063 g/ml), were retained. In the serum ≥1.040 g/ml fraction, TRL and large, buoyant LDL (bLDL) were depleted and sdLDL was retained.

Lipoprotein subfractions in serum and serum fractions were analyzed by cITP on a Beckman P/ACE MDQ system (Beckman-Coulter, Tokyo, Japan) as described previously. Levels of cITP lipoprotein subfractions were expressed as the peak area relative to an internal marker, 5-carboxy-fluorescein.

Homogeneous assays for LDL-C were performed simultaneously in whole serum and serum ≥1.006 g/ml and >1.040 g/ml fractions on an autoanalyzer (Hitachi 7600-0208; Hitachi High-Technologies, Tokyo, Japan) according to the manufacturer’s protocols, as described previously. Reagents for the 2 LDL-C homogeneous assays, that is, the selective elimination method (LDL-C [Se]) and the selective solubilization method (LDL-C [Ky]) were obtained from Sekisui Medical (Tokyo, Japan) and Kyowa Medex (Tokyo, Japan), respectively. The number of LDL and sdLDL particles were measured as apoB concentration in serum ≥1.006 g/ml and >1.040 g/ml fractions, respectively.

Statistical Analysis

Data analysis was performed using SAS version 9.2 (SAS Institute, Cary, NC, USA) at Fukuoka University (Fukuoka, Japan). The Spearman correlation was used to examine the correlation between continuous variables. The equality of 2 population correlations was tested using Fisher’s z Transformation. Regression analysis was performed using the general linear model. Differences in the slopes of regression lines were examined by an analysis of covariance (ANCOVA). LDL-C levels determined by different methods in HC patients were compared by an analysis of variance (ANOVA). The equality of the variance of 2 continuous variables was examined by Bartlett’s test. Significant differences between the direct measurement of LDL-C and LDL-C (F) and the deviation of the differences between 2 continuous variables from zero were examined using the Wilcoxon signed-rank test. Data are presented as mean±SD, and the significance level was set at P<0.05 unless otherwise indicated.

Results

Direct LDL-C Measurement vs. LDL-C (F) in Whole Serum

Both LDL-C (Se) and LDL-C (Ky) were highly correlated with LDL-C (F) in HC patients at baseline (Figure 1A) and after treatment with statins (Figure 1B). The slope of the regression line of LDL-C (Se) vs. LDL-C (F), however, was significantly less than that of LDL-C (Ky) vs. LDL-C (F) (0.77 vs. 0.96, P<0.01) at baseline (Figure 1A). The deviation of LDL-C (Se) from LDL-C (F) was closely and negatively correlated with the deviation of the differences between 2 continuous variables from zero were examined using the Wilcoxon signed-rank test. Data are presented as mean±SD, and the significance level was set at P<0.05 unless otherwise indicated.

The mean values of LDL-C (Se) and LDL-C (Ky) in HC patients at baseline were significantly lower and higher, respectively, than LDL-C (F), and their deviation from LDL-C (F) was negative and positive, respectively (Figure 1E). The variance of the deviation of LDL-C (Se) from LDL-C (F) at baseline was significantly greater than that of LDL-C (Ky) from LDL-C (F) (Figure 1F), as assessed by Bartlett’s test for equal variance.
Figure 1. (A, B) Relationship between low-density lipoprotein cholesterol (LDL-C) measured using 2 homogeneous assays with selective elimination (Sekisui Medical: LDL-C (Se)) and solubilization (Kyorin Medical: LDL-C (Ky)) and LDL-C calculated using the Friedewald formula (LDL-C (F)) in patients with hypercholesterolemia (HC). (A) at baseline and (B) after statin treatment. k, regression coefficient; r, Spearman correlation coefficient. (C, D) Deviations of LDL-C (Se) and LDL-C (Ky) from LDL-C (F) with LDL-C (F) in HC patients (C) at baseline and (D) after statin treatment. (E-a, b) Comparison of LDL-C (Se) and LDL-C (Ky) with LDL-C (F) in HC patients (a) at baseline and (b) after statin treatment. (c, d) Relation of the deviation of LDL-C (Se) and LDL-C (Ky) from LDL-C (F) in HC patients (c) at baseline and (d) after statin treatment. Data are given as mean (SD). *P<0.05 vs. LDL-C (F), (ANOVA); †P<0.05, different from zero (Wilcoxon signed rank test). (Blue) LDL-C (F); (black) LDL-C (Se); (red) LDL-C (Ky). (F) Relationship of the deviation of LDL-C (Se) and LDL-C (Ky) from LDL-C (F) with triglyceride (TG) levels. LDL-C (Ky)–LDL-C (F) was plotted against LDL-C (Se)–LDL-C (F) according to TG strata. (red circles), 2 patients for whom the data on lipoprotein subfractions as analyzed by capillary isoelectric focusing are given in Figure 2.
Factors for Direct LDL-C Measurement Deviation From LDL-C (F) in HC Patients at Baseline

Serum TG levels were positively correlated with the deviation of both LDL-C (Se) and LDL-C (Ky) from LDL-C (F) (Table), but serum levels of TC, non-HDL-C, apoB, and the ratio of RLP-C/TG were negatively correlated with the deviation of LDL-C (Se) from LDL-C (F), while the levels of RLP-C and cITP fLDL were positively correlated with the deviation of LDL-C (Ky) from LDL-C (F) (Table).

To demonstrate the relationship between cITP fLDL and the deviation of LDL-C (Ky) from LDL-C (F), typical electropherograms of 2 HC patients with different deviations of LDL-C (Ky) from LDL-C (F) (patients 1 and 2: –1mg/dl and 12mg/dl) are shown (Figure 2). cITP separated serum lipoproteins according to their electrophoretic mobility into 3 HDL subfraction (fast-, intermediate-, and slow-migrating HDL; fHDL, iHDL, and sHDL), 2 TRL subfractions (fast- and slow-migrating TRL; fTRL and sTRL), and 2 major LDL subfractions (fast- and slow-migrating LDL: fLDL and sLDL; Figures 2A,B). Patient 2 had similar levels of LDL-C (F) to patient 1 (Figure 2C), but had greater fLDL (peak 6) and less sLDL (peak 7) than patient 1 (Figures 2A-a, B-a).

Patient 2 also had markedly increased cITP sTRL (peak 5) in whole serum (Figure 2B-a). In TRL-depleted serum (serum d>1.006 g/ml fraction), patient 2 had markedly elevated very-fast-migrating LDL (vfLDL) (peak 5; Figure 2A-b, B-b), which has the same electrophoretic mobility as sTRL.21,22 We have previously shown that mildly modified LDL is characterized by elevated cITP fLDL, and moderately modified LDL is characterized by both elevated fLDL and vfLDL.23 Therefore, LDL in patient 2 was modified to a greater extent than that in patient 1. Patient 2 also had a different distribution of LDL subfractions in the sLDL fraction and apparently elevated s-ffLDL and s-vfLDL compared to patient 1 (Figures 2A-c, B-C).

Reactivity of Homogeneous LDL Assays Toward LDL Particles and Size- and Charge-based LDL Subfractions

LDL-C (Se) and LDL-C (Ky) were simultaneously measured in the LDL fraction (serum d>1.006 g/ml fraction) and sdLDL fraction (serum d>1.040 g/ml fraction) in HC patients at baseline to examine the reactivity of the 2 direct measurements of LDL-C toward LDL particles and size- and charge-based LDL subfractions (Figure 3). LDL-C (Ky) deviated positively from LDL-C (Se) in the LDL (lbLDL + sdLDL), lbLDL, and sdLDL fractions (Figure 3).

In the LDL fraction, the number of LDL particles measured as LDL-apoB was closely and positively correlated with the deviation of LDL-C (Ky) from LDL-C (Se) (Figure 3A). Although LDL-apoB was closely correlated with both LDL-C (Se) and LDL-C (Ky) (r=0.819 and 0.887, P<0.001), the slope of the regression line of LDL-C (Se) vs. LDL-C (Ky) was significantly lower than that of LDL-C (Ky) vs. LDL-apoB (regression coefficient ±SE: 1.29±0.05 vs. 1.55±0.05, P<0.05), as assessed by ANCOVA (data not shown). The relationship between LDL-apoB and the deviation of LDL-C (Ky) from LDL-C (Se) was not significantly different in lbLDL and sdLDL fractions (Figure 3B).

The cITP cmLDL (vfLDL + fLDL) was positively correlated (P<0.001) with the deviation of LDL-C (Ky) from LDL-C (Se) in LDL (lbLDL + sdLDL), lbLDL, and sdLDL fractions (Figures 3C,D), while sLDL was not correlated with the deviation of LDL-C (Ky) from LDL-C (Se) (Figures 3E,F).

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Table. Factors for Direct LDL-C Measurement Deviation From Calculated LDL-C in Patients With HC†

<table>
<thead>
<tr>
<th>Deviation of LDL-C (Se) from LDL-C (F)</th>
<th>Deviation of LDL-C (Ky) from LDL-C (F)</th>
<th>Difference of LDL-C (Ky) from LDL-C (Se)</th>
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<tr>
<td>r P value</td>
<td>r P value</td>
<td>r P value</td>
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<tr>
<td>TC</td>
<td>–0.454 P&lt;0.001</td>
<td>–0.056 0.35 P&lt;0.001</td>
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<td>TG</td>
<td>0.273 P&lt;0.001</td>
<td>0.350 &lt;0.001</td>
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<td>HDL-C</td>
<td>–0.130 P&lt;0.001</td>
<td>–0.007 0.91 P&lt;0.001</td>
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<tr>
<td>LDL-C (F)</td>
<td>–0.611 P&lt;0.001</td>
<td>–0.243 &lt;0.001</td>
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<tr>
<td>LDL-C (Se)</td>
<td>–0.296 P&lt;0.001</td>
<td>–0.095 0.11 P&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (Ky)</td>
<td>–0.536 P&lt;0.001</td>
<td>–0.028 0.64 P&lt;0.001</td>
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<tr>
<td>Non-HDL-C</td>
<td>–0.399 P&lt;0.001</td>
<td>–0.071 0.24 P&lt;0.001</td>
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<td>ApoA1</td>
<td>–0.025 0.67</td>
<td>0.103 0.08</td>
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<td>ApoB</td>
<td>–0.278 P&lt;0.001</td>
<td>0.082 0.17</td>
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<tr>
<td>ApoE</td>
<td>–0.214 P&lt;0.001</td>
<td>0.182 &lt;0.01</td>
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<tr>
<td>RLP-C</td>
<td>0.022 0.72</td>
<td>0.250 &lt;0.001</td>
</tr>
<tr>
<td>RLP-C/TG</td>
<td>–0.517 P&lt;0.001</td>
<td>–0.07 0.271 P&lt;0.001</td>
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<tr>
<td>cITP fTRL</td>
<td>0.247 P&lt;0.001</td>
<td>0.347 &lt;0.001</td>
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<tr>
<td>cITP sTRL</td>
<td>0.143 P&lt;0.05</td>
<td>0.407 &lt;0.001</td>
</tr>
<tr>
<td>cITP fLDL</td>
<td>0.053 0.37</td>
<td>0.375 &lt;0.001</td>
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<tr>
<td>cITP sLDL</td>
<td>–0.164 &lt;0.01</td>
<td>–0.338 &lt;0.001</td>
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Data given as Spearman correlation coefficients. †At baseline.

LDL-C, low-density lipoprotein cholesterol; HC, hypercholesterolemia; LDL-C (Ky), LDL-C (Se), LDL-C measured using homogenous assays with solubilization (Kyowa Medex) and selective elimination (Sekisui Medical), respectively; LDL-C (F), LDL-C calculated using the Friedewald formula; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; Apo, apolipoprotein; RLP-C, remnant-like particle cholesterol; cITP, capillary isotachophoresis; fTRL, fast-migrating triglyceride-rich lipoprotein (TRL); sTRL, slow-migrating TRL; fLDL, fast-migrating LDL; sLDL, slow-migrating LDL.
Figure 2. (A, B) Lipoprotein subfractions as analyzed by capillary isoelectrophoresis (cITP) in (a) whole serum and (b) serum d>1.006 g/ml fraction and (c) serum d>1.040 g/ml fraction (low-density lipoprotein [LDL] fraction and small, dense LDL [sdLDL] fraction, respectively) in hypercholesterolemic (HC) patients (A) 1 and (B) 2. Peaks 1–3, fast-, intermediate-, and slow-migrating high-density lipoprotein (fHDL, iHDL, and sHDL) subfractions; peaks 4, 5 (a), fast- and slow-migrating triglyceride-rich lipoprotein (fTRL and sTRL) subfractions; peak 5 (b, c), very-fast-migrating LDL (vfLDL) subfraction; peaks 6, 7, fast- and slow-migrating LDL (fLDL and sLDL) subfractions; peak 8, minor LDL subfraction. (C, Left panel) Serum levels of lipids and lipoproteins in HC patients 1 and 2. (Right panel) Levels of LDL-apoB (no. of LDL particles), LDL-C (Se), and LDL-C (Ky) in HC patients 1 and 2 in serum d>1.006 g/ml (Upper) and 1.040 g/ml (Lower) fractions. Units, mg/dl. LDL-C (F), LDL-C calculated using the Friedewald formula; LDL-C (Ky), LDL-C measured using homogenous assays with solubilization (Kyowa Medex) and selective elimination (Sekisui Medical), respectively. RLP-C, remnant-like particle cholesterol.
Figure 3. Relation of the difference between LDL-C (Ky) and LDL-C (Se) to (A,B) LDL-apoB and (C,D) charge-modified LDL (cmLDL: vLDL+fLDL) and (E,F) sLDL subfractions as analyzed by capillary isotachophoresis (cITP) in the (A,C,E) serum d>1.006 g/ml fraction and (B,D,F) LDL density fractions (small, dense LDL [sdLDL] fraction and large, buoyant LDL [lbLDL] fraction) in patients with hypercholesterolemia. Levels of sdLDL-C (Se), sdLDL-C (Ky), sdLDL-apoB, and cITP sdLDL subfractions were measured in the serum d>1.040 g/ml fraction (sdLDL fraction), and lbLDL-C (Se), lbLDL-C (Ky), lbLDL-apoB, and cITP lbLDL subfractions were calculated by subtracting sdLDL-C (Se), sdLDL-C (Ky), sdLDL-apoB, and cITP sdLDL subfractions measured in the serum d>1.040 g/ml fraction from LDL-C (Se), LDL-C (Ky), LDL-apoB, and cITP LDL subfractions measured in the serum d>1.006 g/ml fraction, respectively. The plot of the deviation of sdLDL-C (Ky) from sdLDL-C (Se) vs. sdLDL-apoB (orange) and the plot of the deviation of lbLDL-C (Ky) from lbLDL-C (Se) vs. lbLDL-apoB (green) were overlaid and presented together (this doubles the number of data points). r, Spearman correlation coefficient. LDL-C (F), low-density lipoprotein cholesterol (LDL-C) calculated using the Friedewald formula; LDL-C (Ky), LDL-C (Se), LDL-C measured using homogenous assays with solubilization (Kyowa Medex) and selective elimination (Sekisui Medical), respectively.
Discussion

The measurement of LDL-C is important for assessing the risk of CHD and evaluating the effects of cholesterol-lowering therapy. Modified forms of LDL including sLDL and electronegative LDL (cmLDL) are atherogenic.\textsuperscript{11,16} We compared the 2 most widely used homogeneous assays for LDL-C in terms of their relationship to LDL-C (F) in whole serum and their reactivity to sLDL and cmLDL as analyzed by cITP in serum ultracentrifugation fractions in HC patients.

We found that the relationships between the 2 direct measurements of LDL-C and LDL-C (F) were different in HC patients at baseline (Figure 1A), but were not significantly different after statin treatment (Figure 1B) when LDL-C was reduced (Figure 1E). We have previously shown that statin treatment effectively reduces both charge-based and size-based LDL subfractions and RLP-C.\textsuperscript{18,21,26} This agrees with the Miller et al study, which stated that the deviation of the direct measurement of LDL-C from ultracentrifugation RMP was greater in diseased patients than in non-diseased patients.\textsuperscript{4}

At baseline, LDL-C (Se) and LDL-C (Ky) (Ky) were significantly lower and higher, respectively, than LDL-C (F) (Figure 1E). After treatment with statins, the negative deviation of LDL-C (Se) from LDL-C (F) and the positive deviation of LDL-C (Ky) from LDL-C (F) in HC patients were not significantly different from zero (Figure 1E). The median percent reductions in LDL-C (F), LDL-C (Se), and LDL-C (Ky) in HC patients after statin treatment were similar (45%, 43%, and 45%, respectively, data not shown). Therefore, although the method used to measure LDL-C could be important when assessing the individual risk for CHD, it should be less of a problem when evaluating the effects of cholesterol-lowering therapy.\textsuperscript{30-33}

We found that the factors that contribute to the deviations of the 2 direct LDL-C measurements from LDL-C (F) were also different (Table). The novel finding is that the deviation of LDL-C (Ky) from LDL-C (F) in HC patients at baseline was positively correlated with the LDL subfraction as analyzed by cITP (Table). To understand this, we presented the examples of 2 HC patients who showed no deviation and a positive deviation, respectively, of LDL-C (Ky) from LDL-C (F) (patients 1, 2; Figure 2). Although patients 1 and 2 had similar LDL-C (F), they had different distributions of charge-based LDL subfractions as analyzed by cITP, patient 2, who had a positive deviation of LDL-C (Ky) from LDL-C (F), had a much higher level of cmLDL (cITP vLDL and iLDL), a more atherogenic lipoprotein profile, compared to patient 1, who had no deviation of LDL-C (Ky) from LDL-C (F) (Figure 2).

Previous studies have compared different homogeneous methods for determining LDL-C in whole plasma or serum with regard to their reactivities toward lipoproteins,\textsuperscript{45,23} and in pure LDL separated with ultracentrifugation with regard to their reactivities toward LDL.\textsuperscript{34} We compared the reactivities of 2 homogeneous assays for LDL-C toward LDL particles in the serum d>1.006 g/ml fraction (LDL fraction) and d>1.040 g/ml fraction (sLDL fraction), because serum proteins protect LDL from oxidative modification during separation and analysis, and quantitative results can be obtained. We are the first to compare the reactivities of 2 homogeneous assays toward LDL size- and charge-based LDL subfractions in HC patients.

We found a good correlation between LDL-C (Se) or LDL-C (Ky) measured in the LDL fraction and the number of LDL particles measured as LDL-apoB, but a lower slope for the regression line of LDL-C (Se) vs. LDL-apoB (data not shown). The deviation of LDL-C (Ky) from LDL-C (Se) was positive and closely correlated with LDL-apoB (Figure 3A). This indicates that LDL-C (Ky) more accurately reflects the number of LDL particles and LDL-C (Se) was less reactive toward LDL particles.

The reactivities of the 2 homogeneous assays toward sLDL and cmLDL were similar (Figure 3B), but we found that the LDL-C (Ky) assay was also more reactive toward cmLDL than the LDL-C (Se) assay, because the deviation of LDL-C (Ky) from LDL-C (Se) was positively and significantly correlated with cmLDL, but not with sLDL in the LDL, iLDL, and sLDL fractions (Figures 3C–F).

The discrepancy between the 2 homogeneous assays for LDL-C that we observed in HC patients at baseline, but not after statin treatment when LDL particles were reduced (Figure 1), may be attributed to 2 factors. First, direct LDL-C assays have not been adequately standardized because normolipidemic specimens, but not unusual specimens, are typically used for standardization.\textsuperscript{35} The manufacturers assign calibration values that optimize the agreement with the reference method.\textsuperscript{38} Therefore, greater variations may be observed in patients with dyslipidemia. Second, the LDL-C (Se) assay and LDL-C (Ky) assay use different mechanisms to measure LDL-C levels.\textsuperscript{21,23} The LDL-C (Se) assay measures LDL-C with a surfactant after the elimination of non-LDL cholesterol with a surfactant and an enzyme reaction,\textsuperscript{33} and the LDL-C (Ky) assay measures LDL-C selectively with a surfactant while blocking non-LDL cholesterol with a surfactant and polyamion. These 2 direct LDL-C assays may not measure the same LDL fractions.\textsuperscript{35,36} The LDL-C (Ky) assay may include more of the remnants with LDL (Table) and more cmLDL (Figure 3), which is correlated with RLP-C,\textsuperscript{18,21,26} while the LDL-C (Se) assay may be more specific for narrow-cut LDL and suppresses the measurement of remnant lipoprotein cholesterol (Table) and cmLDL (Figures 3).

In the current guidelines for the management of dyslipidemia and the prevention of cardiovascular disease, non-HDL-C is recommended as a secondary target for lipid-lowering therapy in patients with high TG (≥200 mg/dl). Van Deventer et al reported that non-HDL-C is more accurate than direct and calculated LDL-C for estimating cardiovascular risk in dyslipidemic samples.\textsuperscript{37} Direct HDL-C assays, however, are also problematic, and the bias and imprecision associated with HDL-C measurement affect the reliability of the non-HDL-C calculation.\textsuperscript{4,38} In addition, VLDL particles are not as atherogenic as LDL particles.

The specific measurement of LDL-C and HDL-C is challenging because LDL and HDL are heterogeneous particles that differ with respect to lipid and protein composition, size, and electric charge. ApoB level has been shown to be a good marker of the LDL particle concentration.\textsuperscript{38} Because apoB is a well-characterized protein, apoB measurement might possibly be an alternative to LDL-C measurement.

In conclusion, 2 of the most widely used homogeneous assays for LDL-C, the LDL-C (Se) assay and LDL-C (Ky) assay, differ with regard to their reactivity toward LDL particles and cmLDL in HC patients. Direct measurement of LDL-C that reflects the number of LDL particles and cmLDL could be a better marker for the risk of CHD. Further investigation is needed to determine whether the inclusion of cmLDL in LDL-C measurement is related to clinical outcome.

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