Utility of Direct Measurement of Low-Density Lipoprotein Cholesterol in Dyslipidemic Pediatric Patients

Baruch S. Ticho, MD, PhD; Ellis J. Neufeld, MD, PhD; Jane W. Newburger, MD, MPH; Neil Harris, MD; Annette Baker, RN; Nader Rifai, PhD

Background: Low-density lipoprotein cholesterol (LDL-C) levels are the primary basis for treatment guidelines established for hyperlipidemic children and adolescents. Levels of LDL-C are commonly monitored by means of the Friedewald formula, an indirect calculation that requires an overnight fast. A new method has been developed for the direct measurement of LDL-C (DLDL-C) that does not require fasting. We evaluated the clinical utility of this method.

Design: We determined LDL-C concentrations simultaneously by the DLDL-C method, Friedewald equation, and β-quantification (reference procedure).

Setting: Pediatric dyslipidemia clinic at Children’s Hospital, Boston, Mass.

Patients: Ninety-two fasting hyperlipidemic pediatric patients.

Results: At the LDL-C concentration cutoffs commonly used for making therapeutic decisions, the DLDL-C method had a significant negative bias (P ≤ .05) and misclassified patients into incorrect treatment groups more often than the Friedewald method. The negative predictive value for the DLDL-C method was lower than that for the Friedewald method (P ≤ .05), and the cost of determining LDL-C level with the new method was 3 times greater.

Conclusions: The misclassification potential for LDL-C, and the assay costs, were greater for the DLDL-C method than for the Friedewald calculation. Despite the apparent advantages of the DLDL-C method, we conclude that for hyperlipidemic children the utility of this new method is not advantageous over the conventional Friedewald method. In some conditions, such as in diabetes or marked hypertriglyceridemia, the DLDL-C method may be useful.


Editor’s Note: It’s nice to document that newer doesn’t necessarily mean better, especially when it’s more expensive.

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COMPPELLING evidence demonstrates that the atherosclerotic process begins in childhood and progresses slowly into adulthood, at which time it leads frequently to coronary heart disease, one of the major causes of death in the United States. Increased concentrations of plasma cholesterol and low-density lipoprotein cholesterol (LDL-C) and decreased concentrations of high-density lipoprotein cholesterol (HDL-C) are important independent risk factors for the development of coronary heart disease. In 1992 the National Cholesterol Education Program (NCEP) Expert Panel on Blood Cholesterol Levels in Children and Adolescents established guidelines for detecting, evaluating, and treating pediatric patients with high concentrations of total cholesterol and LDL-C. Classification levels for acceptable, borderline, and high total cholesterol and LDL-C concentrations were established for children and adolescents from families with hypercholesterolemia or premature cardiovascular or cerebrovascular disease. As a result of NCEP screening recommendations, and subsequent recommendations from the American Academy of Pediatrics, an increasing number of children with abnormally high lipid concentrations are being identified. A confirmed high LDL-C level leads to a clinical evaluation and clinical intervention. For severely affected individuals, this may include professional dietary counseling and possible pharmacological treatment. Monitoring and follow-up, which involve the assessment of dietary compliance and lipid levels, are important components of successful therapy in patients with hyperlipidemia. Thus, repeated measurement of

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PATIENTS AND METHODS

STUDY PATIENTS

The study population included 92 hyperlipidemic children and adolescents who were referred to a hospital-based lipid clinic. More than 70% of patients had a family history of premature heart disease, and nearly all patients had a family history of hypercholesterolemia. Most patients had familial combined hyperlipidemia. All patients were younger than 21 years and had triglyceride levels less than 4.52 mmol/L (400 mg/dL). Samples were collected during a 6-month period. At the time of blood collection, all patients were healthy and had been fasting for a minimum of 12 hours. This study protocol was approved by the institutional review board of Children’s Hospital, Boston, Mass.

BIOCHEMICAL ANALYSES

Blood samples were collected into evacuated heparinized tubes after a 12-hour fast. Levels of total cholesterol, HDL-C, and triglycerides were determined enzymatically (Hitachi 911 analyzer; Boehringer-Mannheim, Indianapolis, Ind). The samples were analyzed during a 6-month period with the use of more than 20 analytical runs. Triglyceride measurement was corrected for the presence of endogenous glycerol. The HDL was separated by precipitation technique with dextran sulfate and magnesium chloride as previously described.

Total cholesterol level was determined with a day-to-day variation of 1.3% at concentrations of 3.63 mmol/L (140 mg/dL) and 5.18 mmol/L (200 mg/dL); coefficients of variation for triglycerides were 2.0% and 1.6% for concentrations of 1.13 mmol/L (100 mg/dL) and 2.26 mmol/L (200 mg/dL), respectively; and coefficients of variation for HDL-C were 2.7% and 2.0% at concentrations of 0.62 mmol/L (24 mg/dL) and 1.24 mmol/L (48 mg/dL), respectively. Total cholesterol level was determined with a mean ± SD bias from the Centers for Disease Control and Prevention target value of 0.62% ± 1.11% for concentrations ranging from 3.31 to 6.10 mmol/L (128 to 236 mg/dL); triglyceride average ± SD bias of −0.89% ± 3.12% for concentrations ranging from 0.61 to 2.64 mmol/L (54 to 234 mg/dL); and HDL-C average ± SD bias of −1.01% ± 2.40% for concentrations ranging from 0.70 to 2.05 mmol/L (27 to 79 mg/dL). Our laboratory is certified by the National Heart, Lung, and Blood Institute and Centers for Disease Control and Prevention Lipid Standardization Program.

The LDL-C level was determined by 3 methods: Friedewald calculation, DLDL-C, and β-quantification. The Friedewald calculation determines LDL-C level by the following equation: LDL-C = total cholesterol − (HDL-C + triglycerides/5), where triglycerides/5 is an estimate of very-low-density lipoprotein cholesterol and all concentrations are expressed in milligrams per liter. When values are expressed in millimoles per liter, very-low-density lipoprotein cholesterol is estimated as triglycerides/2.22. The DLDL-C was performed with reagents (Sigma Diagnostics, St Louis, Mo) according to the manufacturer’s instructions. The β-quantification, which involves ultracentrifugation and a precipitation step, was performed as described previously. The same total cholesterol and HDL-C measurements were used to calculate both the Friedewald and β-quantification LDL-C levels.

STATISTICAL ANALYSES

The bias for each measurement was calculated by determining the difference between the test method, in this case the DLDL-C or Friedewald, and the reference procedure, the β-quantification.

The positive predictive value of an LDL-C assay at each specified cutoff point was calculated as [true positive/(true positive + false positive)] × 100, where true positive means that LDL-C results of both the reference method and the test method are greater than or equal to the cutoff concentration, and false positive means that the test method LDL-C result is greater than the cutoff when the reference method LDL-C is less than the cutoff. The negative predictive value at each specified cutoff point was calculated as [true negative/(true negative + false negative)] × 100, where true negative means that LDL-C results of both the reference method and the test method are less than the cutoff concentration, and false negative means that the test method LDL-C result is less than the cutoff concentration when the reference method LDL-C is greater than or equal to the cutoff concentration. Statistical significance was determined with Fisher exact test.

COST ANALYSIS STUDY

Labor cost was established by the actual amount of time it took to run 20 samples, with a technologist’s hourly wage of $20. Instrument cost was determined by adding the cost of the analyzer to that of the service maintenance, which was averaged over the expected life of the instrument (5 years) and divided by the number of all tests performed annually on that particular autoanalyzer (500 000). The cost of the reagents was determined by multiplying the cost per test by 1.2 to account for the reagents used for calibration, quality control, and repeats.

Blood lipid concentrations is used to assess the success of dietary intervention and the need for medication.

Although there are numerous laboratory procedures with varying complexity for investigating lipid disorders, most dyslipidemic patients are routinely examined by means of the measured total cholesterol, HDL-C, and triglyceride concentrations and the calculated LDL-C concentration by the Friedewald formula. This estimation of LDL-C level can only be performed in patients who have fasted for 12 hours and have triglyceride levels less than 4.52 mmol/L (400 mg/dL). This extended fast is a particular hardship for children, and the need for early-morning visits for blood tests can be onerous for pediatricians’ offices to arrange.

Recently, a direct LDL-C (DLDL-C) method that does not require a fasting specimen has been developed. This technique involves the removal of very-low-density lipoproteins and HDL from serum by immunoprecipitation, using specific anti-apolipoprotein A-I and anti-apolipoprotein E antibodies. The LDL-C concentration is then measured directly in the sample by a standardized enzymatic cholesterol assay. Several recent studies...
Table 1. Characteristics of the Patients Enrolled in the Study*

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>11.6 ± 3.8</td>
<td>4-20</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L (mg/dL)</td>
<td>6.06 ± 1.5 (234 ± 57)</td>
<td>3.73-9.19 (144-355)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L (mg/dL)</td>
<td>0.96 ± 0.23 (37 ± 9)</td>
<td>0.39-1.47 (15-57)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L (mg/dL)</td>
<td>1.20 ± 0.82 (107 ± 73)</td>
<td>0.16-1.84 (14-130)</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L (mg/dL)</td>
<td>4.40 ± 1.45 (170 ± 56)</td>
<td>1.79-7.74 (69-299)</td>
</tr>
<tr>
<td>β-Quantification</td>
<td>4.40 ± 1.45 (170 ± 56)</td>
<td>1.79-7.74 (69-299)</td>
</tr>
<tr>
<td>Direct</td>
<td>4.17 ± 1.42 (161 ± 55)</td>
<td>1.48-7.64 (57-295)</td>
</tr>
<tr>
<td>Friedewald</td>
<td>4.56 ± 1.45 (176 ± 56)</td>
<td>1.81-7.98 (70-308)</td>
</tr>
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* HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.

Figure 1. Measurement bias of the direct low-density lipoprotein cholesterol (DLDL-C) and Friedewald methods vs the β-quantification reference method.

have demonstrated the reasonable accuracy of the DLDL-C method in hyperlipidemic adults\(^\text{10,11}\) and children.\(^\text{12}\) However, controversy remains regarding the clinical utility of this assay.\(^\text{12,14}\)

In this study, we compared LDL-C levels determined by the Friedewald calculation and the DLDL-C assay with the β-quantification method, which has been accepted as the reference method for LDL-C determination by the NCEP Working Group on Lipoprotein Measurement,\(^\text{15}\) in a pediatric hyperlipidemic population. This comparison allowed us to assess the clinical utility of the DLDL-C method in children observed in a lipid clinic.

RESULTS

A total of 92 samples were collected from fasting patients. The characteristics of the study population are shown in Table 1. As expected, the mean total cholesterol, LDL-C, and triglyceride concentrations were above the 95th percentile values for the general pediatric population.

Previous studies have shown that the DLDL-C assay meets the guidelines for precision with regard to within-run and run-to-run coefficients of variation, and the accuracy of the DLDL-C is comparable with that of the Friedewald method in adults\(^\text{10,11}\) and children.\(^\text{12}\) Using the data presented herein, we determined the biases for each measurement by each method and a best-fit line for the plot was obtained (Figure 1). The DLDL-C has a negative bias for all LDL-C levels above approximately 1.94 mmol/L (75 mg/dL) (\(P < .05\)). The Friedewald method has a small, overall positive bias for LDL-C levels less than approximately 7.12 mmol/L (275 mg/dL), which is not statistically significant.

The DLDL-C method and the Friedewald method were compared for the ability to appropriately classify patients into treatment groups as established by the NCEP (dietary therapy: suggested for LDL-C level \([\geq 3.37 \text{ mmol/L} \ (130 \text{ mg/dL})]\); consideration of pharmacologic therapy: either LDL-C level \(> 4.92 \text{ mmol/L} \ (> 190 \text{ mg/dL})\) or LDL-C level \(\geq 4.14 \text{ mmol/L} \ (\geq 160 \text{ mg/dL})\) plus 2 or more other risk factors). The results are shown as percentages in Table 2 and Table 3. In the LDL-C group between 3.37 mmol/L (130 mg/dL) and 4.92 mmol/L (190 mg/dL), only one half of patients were correctly classified by the DLDL-C method, compared with three fourths by the Friedewald method.

We calculated the positive and negative predictive values for each method as compared with the reference procedure (Figure 2). These values were determined by means of cutoff levels of LDL-C concentration based on the NCEP guidelines of 3.37 mmol/L (130 mg/dL),

Table 2. Samples Correctly Classified for LDL Cholesterol According to NCEP Guidelines: Direct LDL vs β-Quantification Method*

<table>
<thead>
<tr>
<th>LDL-C Range</th>
<th>Friedewald LDL</th>
<th>Direct LDL</th>
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<tr>
<td>&lt;3.37</td>
<td>28/32 (88)</td>
<td>26/32 (81)</td>
</tr>
<tr>
<td>3.37-4.13</td>
<td>10/22 (45)</td>
<td>13/22 (60)</td>
</tr>
<tr>
<td>4.14-4.91</td>
<td>1/22 (5)</td>
<td>0/22 (0)</td>
</tr>
<tr>
<td>≥4.92</td>
<td>1/22 (5)</td>
<td>0/22 (0)</td>
</tr>
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* LDL indicates low-density lipoprotein; NCEP, National Cholesterol Education Program. Values are given as number of samples so classified per total number (percentage); boldface values show correctly classified samples.

Table 3. Samples Correctly Classified for LDL Cholesterol According to NCEP Guidelines: Friedewald LDL vs β-Quantification Method*

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<tr>
<th>LDL-C Range</th>
<th>Friedewald LDL</th>
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</thead>
<tbody>
<tr>
<td>&lt;3.37</td>
<td>16/24 (67)</td>
<td>19/24 (79)</td>
</tr>
<tr>
<td>3.37-4.13</td>
<td>2/24 (8)</td>
<td>0/24 (0)</td>
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significant clinical decisions must be based on arbitrary values near the NCEP cutoff values. The Friedewald method for approximating LDL-C concentration is convenient for regular clinical use, and it provides a remarkably good estimate of the LDL-C level in the ranges of interest. However, the Friedewald method may not meet the standards for precision and accuracy established by NCEP guidelines. It also requires a fasting sample and is unreliable for samples with triglyceride levels greater than 4.52 mmol/L (400 mg/dL), samples with chylomicrons, and samples from patients with type III hyperlipidemia.

In evaluating the new direct method for LDL-C determination, we found, somewhat to our surprise, that in hyperlipidemic children the DLDL-C method has significant negative bias at LDL levels greater than 3.37 mmol/L (130 mg/dL). As a result, the negative predictive value of the DLDL-C method is less than that of the Friedewald method (ie, a greater percentage of patients were incorrectly classified as unqualified for therapy, or “negative” as compared with the measurements by the Friedewald method). In addition, the cost of the DLDL-C assay is 3 times greater than that of the Friedewald method. The question arises whether the convenience of not fasting is worth the significantly greater cost of performing the nonfasting test.

Overall, we conclude that the DLDL-C is advantageous for use in the following groups of patients: (1) Patients with triglyceride levels above 4.52 mmol/L (400 mg/dL), because the Friedewald LDL-C level is not calculable in this range. This group constitutes approximately 1% of our pediatric lipid clinic patient population, but it would be a larger fraction of adults, or patients with diabetes or significant obesity. The method has been evaluated previously in hypertriglycerideremic pediatric patients. (2) Patients taking lipid-lowering medications,

Guidelines established by the NCEP recommend treatment of children and adolescents with hyperlipidemia with LDL-C measurements and cutoffs for therapy of 3.37 mmol/L (130 mg/dL), 4.14 mmol/L (160 mg/dL), and 4.92 mmol/L (190 mg/dL). The cutoffs were established by the NCEP “expert panel” after consideration of the available literature, particularly the population norms established by the Population Studies of the Lipid Research Clinics. Although following the guidelines appears simple enough, significant clinical decisions must be based on arbitrary cutoffs. If the guidelines were followed to the letter, the characteristics of the LDL-C testing method would be critical in determining whether a patient was referred to a dietitian or received cholesterol-lowering medication. Lipid Research Clinic reference values were determined by β-quantification, though the method is rarely used today except in research studies. To the extent that a new testing method differs from the Lipid Research Clinic methods and population values, the guidelines would need to change accordingly or become less useful.

We have compared the Friedewald (approximation) method with a new assay specific for LDL-C in the setting of a pediatric lipid clinic, where, for the majority of patients, decisions must be made on the basis of lipid values near the NCEP cutoff values. The Friedewald method for approximating LDL-C concentration is convenient for regular clinical use, and it provides a remarkably good estimate of the LDL-C level in the ranges of interest. However, the Friedewald method may not meet the standards for precision and accuracy established by NCEP guidelines. It also requires a fasting sample and is unreliable for samples with triglyceride levels greater than 4.52 mmol/L (400 mg/dL), samples with chylomicrons, and samples from patients with type III hyperlipidemia.

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who have normal triglyceride and HDL-C levels and who need only routine follow-up of LDL-C levels. Most of these subjects will have LDL-C levels greater than 4.92 mmol/L (190 mg/dL), a range where the DLDL-C is very reliable. In our pediatric lipid practice, this group comprises at most 15% of the patient population. (3) Patients for whom fasting is contraindicated or a significant hardship (eg, diabetic patients or young children).  

In contrast, we find that the DLDL-C is not advantageous for use in the following groups: (1) patients with LDL-C levels between 3.37 and 4.92 mmol/L (130 and 190 mg/dL) (a prevalent group of young persons under consideration for therapy), because the DLDL-C is insufficiently reliable in this range to allow for decisions regarding possible pharmacological intervention; (2) patients with mild to moderate hypertriglyceridemia (triglyceride levels between 1.13 and 4.52 mmol/L [100 and 400 mg/dL]), because these individuals would still need fasting samples drawn as part of routine follow-up to monitor triglyceride levels; and (3) patients undergoing initial evaluation, because fasting triglycerides must be measured to assess the lipid phenotype. We conclude that, for hyperlipidemic patients without hypertriglyceridemia, the utility of the new DLDL-C method is not superior to, and may be inferior to, that of the conventional Friedewald method.

The question arises: How can the specific DLDL-C determination method give an unsatisfactory result, if it is based on specific precipitation of non-HDL lipid particles? The answer lies in the method for β-quantification, which also calls for a precipitation step, by chemical means rather than being antibody based. We postulate, on the basis of the negative bias of the DLDL-C assay at lower LDL-C concentrations, that a small population of lipoprotein, possibly intermediate-density lipoprotein, is removed from the LDL fraction in the DLDL-C assay during precipitation but not from the β-quantification method. This possibility is the subject of future studies. If this is the case, the DLDL-C assay is not “wrong,” since it in fact selectively measures LDL-C. However, this may reduce the usefulness of the DLDL-C measurement because it would omit some of the atherogenic particles included in the Friedewald and β-quantification methods. Since the treatment guidelines of the NCEP are based on Lipid Research Clinic normal values using the β-quantification method, the LDL-C values obtained with the new method lead to more frequent misclassification than with the old method. Thus, the DLDL-C assay has limited utility in hyperlipidemic young people.

References


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