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Original

Low-density Lipoprotein Cholesterol Measurement Using the Direct Method Versus the Friedewald Equation, and the Clinical Background

Hiroko Kanno,[1,2] **Hitomi Miura,**[3] and **Asako Sato**[1,2]

[1]Department of Clinical Laboratory, Tokyo Women's Medical University School of Medicine, Tokyo, Japan
[2]Department of Diabetology and Metabolism, Tokyo Women's Medical University School of Medicine, Tokyo, Japan
[3]Center of Clinical Laboratory, Tokyo Women's Medical University Hospital, Tokyo, Japan

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Aim: We aimed to compare the low-density lipoprotein cholesterol (LDL-c) values obtained by direct measurement (D) and by the Friedewald equation (F), and examine the validity of the LDL-c + 30 mg/dL value as a non-high-density lipoprotein cholesterol (non-HDL-c) reference value. Additionally, we evaluated the association between the discrepancies between D and F and the clinical background.

Methods: We collected 2,237 samples from patients ≥ 20 year-old, in either fasting or non-fasting state.

Results: The Spearman's correlation coefficient between D and F was 0.964 and there was a correlation between LogD and LogF (R^2 = 0.9259). The average of % Bias was −4.94% in TG < 400 mg/dL. A weak correlation between non-HDL-c and D + 30 was observed in TG ≥ 400 mg/dL (R^2 = 61%). In the most cases with D lower than F, end-stage liver disease was observed. In the cases with D higher than F, no particular diseases were observed.

Conclusion: In conclusion, a significant correlation was found between D and F. Both D and F could be continuously compared and examined as follow-up data in TG < 400 mg/dL. We proposed to reconsider LDL-c + 30 mg/dL as a reference value of non-HDL-c in TG ≥ 400 mg/dL.

Keywords: low-density lipoprotein cholesterol, direct LDL-c assay, Friedewald equation

Introduction

Dyslipidemia is an important clinical risk factor for atherosclerotic disease. In particular, a high concentration of serum low-density lipoprotein cholesterol (LDL-c) increases the risk of morbidity and mortality of coronary heart disease (CHD).[1-4] When the Japan Atherosclerosis Society (JAS) decided to use the LDL-c value as an indicator for the prevention of atherosclerotic disease in 2007, it accepted the LDL-c values calculated by both the Friedewald equation and direct measurement[5] using homogeneous assays. However, there were some reports that differences in LDL-c values were observed among homogeneous asseys.[6-8] Thus, in 2012, the JAS changed their recommendation such that the LDL-c value was defined as only the value calculated from the Friedewald

Corresponding Author: Asako Sato, Department of Clinical Laboratory, Tokyo Women's Medical University School of Medicine, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan. asako.sato@twmu.ac.jp
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equation. In the case of triglyceride (TG) levels ≥ 400 mg/dL, non-high-density lipoprotein cholesterol (non-HDL-c) was used as an indicator instead of LDL-c. The reference value of non-HDL-c was the reference value of LDL-c plus 30 mg/dL.[9-11]

Owing to these changes in the evaluation of the LDL-c value, many clinicians have been concerned about whether these LDL-c data could be used as follow-up data over the past several years. Therefore, we aimed to compare the LDL-c values obtained by direct measurement (D) and by the Friedewald equation (F) in TG < 400 mg/dL, and whether there is continuity between D and F. We also compared the value of non-HDL-c and LDL-c + 30 mg/dL in TG ≥ 400 mg/dL. Furthermore, we attempted to reveal the association between the data discrepancies between these two methods even in TG < 400 mg/dL and the clinical background.

Materials and Methods

Subjects

This cross-sectional study was conducted at the Tokyo Women's Medical University Hospital. The enrollment criteria of the samples from the patients were followed; inpatients or outpatients of Tokyo Women's Medical University Hospital, ordered to measure four lipid data: total cholesterol (TC), TG, LDL-c on the specified two days in November 2013, patients aged 20 years and older, and the cases who did not show the intention of the consent withdrawal by the opt-out. A total of 2,237 consecutive samples were collected from patients. The study was approved by the Ethics Committee of the Tokyo Women's Medical University (No. 3537).

Methods

We evaluated the following relationships: (1) the correlation between D and F in TG < 400 mg/dL; (2) the correlation between non-HDL-c and LDL-c + 30 mg/dL in TG ≥ 400 mg/dL; (3) the association between the data discrepancies between D and F even in TG < 400 mg/dL and the clinical background.

Samples were collected under fasting or non-fasting conditions in plastic vacuum tubes (Venoject II®; Terumo, Tokyo, Japan). These blood samples were centrifuged at 3,300 x g for 6 min at 4°C to prepare the serum samples within 24 h, and all lipid components, TC, TG, high-density lipoprotein cholesterol (HDL-c), and D were measured using an automatic analysis equipment LABOSPECT 008 (Hitachi High-Technologies clinical analyzer, Tokyo, Japan). Determiner L TC II®, Determiner L TG II®, Metabolead HDL-C®, and Determiner L LDL-C® (Kyowa Medex, Tokyo, Japan) were used for the homogeneous assay. LDL-c was calculated using the Friedewald equation as follows: LDL-c = TC − HDL-c − TG/5. Non-HDL-c was calculated as follows: non-HDL-c = TC − HDL-c.

We derived the patients' diagnoses from hospital medical records and investigated whether the diseases affected the differences between D and F.

Statistical analysis

All analyses were conducted using SPSS version 22 (IBM SPSS Statistics Inc., Chicago, Illinois, USA). Data are expressed as mean ± standard deviation (SD) or as frequencies (percentages).

In the range of TG < 400 mg/dL, the correlation between D and F were assessed using Spearman's correlation coefficient and the regression analysis using log transformation data (LogD and LogF). The data discrepancies between D and F were assessed by % bias: (F − D) / D. In the range of TG ≥ 400 mg/dL, the correlation between non-HDL-c and D + 30 were assessed using simple linear regression analysis using an analysis of variance (ANOVA). For all analyses, p-value of <0.05 was considered statistically significant.

Results

We collected 2,237 samples from 1,230 male and 1,007 female patients with a mean age of 55 ± 16 years in either the fasting or non-fasting state. The lipid profiles are shown in **Table 1**. The mean values of D and F were 108 ± 33 mg/dL and 101 ± 32 mg/dL, respectively. The serum TG ranged from 21 mg/dL to 1,112 mg/dL. The samples which TG was < 400 mg/dL were 2,199, and which TG ≥ 400 mg/dL were 38.

In the range of TG < 400 mg/dL, the Spearman's correlation coefficient between D and F was 0.964. The correlation between LogD and LogF was LogD = 0.9946 ×

LogF + 6.9959 (R^2 = 0.9259) (**Figure 1**). Furthermore, the average of % Bias was −4.94% (minimum value −97%, maximum value 1,010%).

In the range of TG ≥ 400 mg/dL, non-HDL-c is recommended to use instead of the Friedewald equation, only a weak correlation between non-HDL-c and D+30 was observed (R^2 = 61%) (**Figure 2**).

To reveal the cause of discrepancies between D and F in TG < 400 mg/dL, the clinical backgrounds are investigated. The 2,199 samples were placed in order of the value of the difference between D and F. After arranging them in this manner, samples were divided into groups that were higher and lower than the 95th percentile from the median value, and their medical records were investigated for diseases (data not shown). There were nine samples in the group lower than the 95th percentile, which meant that D was lower than F. Six out of these nine samples were diagnosed as end-stage liver cancer and severe liver failure. There were 128 samples in the group that were higher than the 95th percentile, which meant that D was higher than F. In this group, dyslipidemia, hypertension, diabetes, and other non-specific diseases were observed. These samples did not include specific cancers or liver cancer.

Table 1. Lipid profiles of all samples.

	mean	SD	minimum	maximum
D (mg/dL)	108	33	2	262
F (mg/dL)	101	32	-5	247
TC (mg/dL)	187	41	35	396
TG (mg/dL)	131	91	21	1,112
HDL-c (mg/dL)	60	20	5	163
non-HDL-c (mg/dL)	127	37	26	292
D + 30 (mg/dL)	138	33	32	292

D, direct measurement of low-density lipoprotein cholesterol; F, calculated low-density lipoprotein cholesterol using the Friedewald equation; TC, total cholesterol; TG, triglyceride; HDL-c, high-density lipoprotein cholesterol; non-HDL-c, non-high-density lipoprotein cholesterol; D + 30, direct measurement of low-density lipoprotein cholesterol + 30; SD, standard deviation.

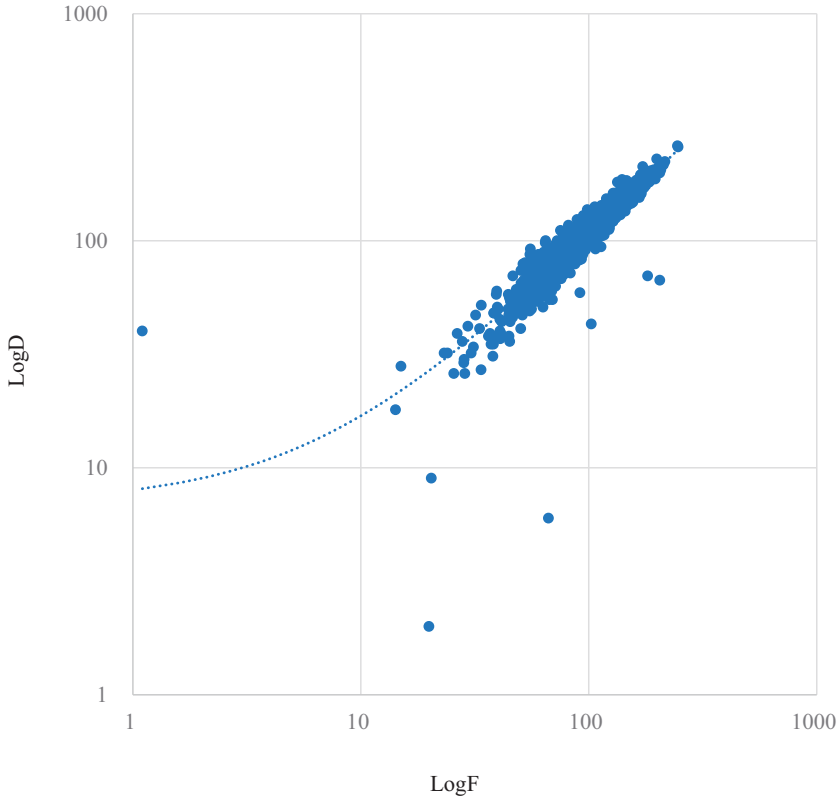

Figure 1. Relationship of the log transformation value between direct measurement of LDL-cholesterol and calculated LDL-cholesterol using the Friedewald equation in the range of < 400 mg/dL triglyceride (n = 2,199).
LogD = 0.9946 × LogF + 6.9959 (R^2 = 0.9259, p < 0.001)
D, direct measurement of low-density lipoprotein cholesterol; F, calculated low-density lipoprotein cholesterol as per the Friedewald equation.

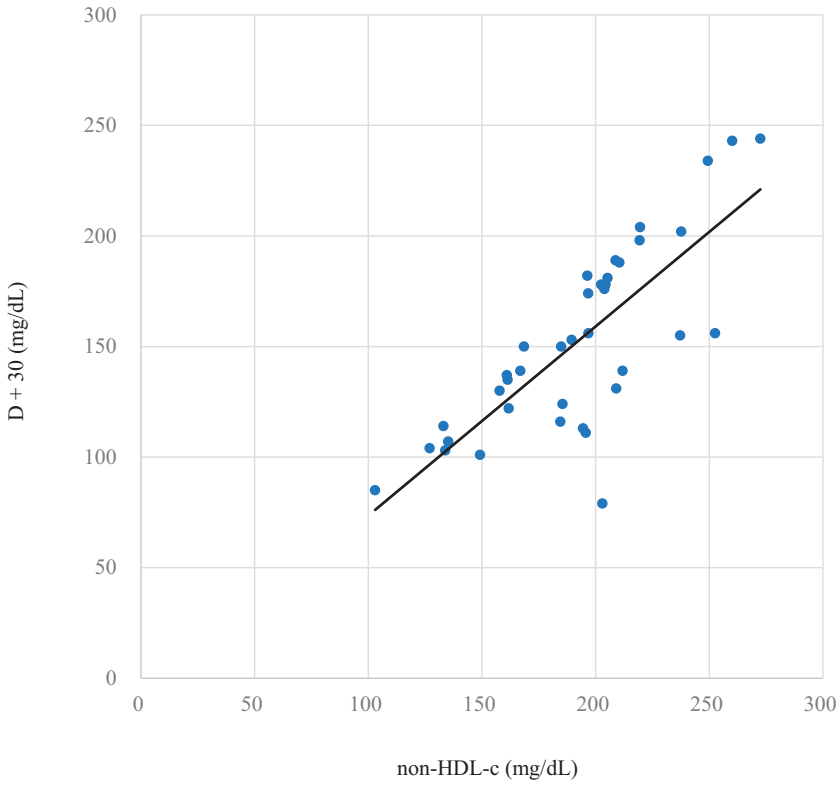

Figure 2. Relationship between non-high-density lipoprotein cholesterol and direct measurement of low-density lipoprotein cholesterol + 30 mg/dL in the range of ≥ 400 mg/dL triglyceride (n = 38).
$y = 0.855\ x - 11.928$ ($R^2 = 61\%$, $p < 0.001$)
D, direct measurement of low-density lipoprotein cholesterol; non-HDL-c, non-high-density lipoprotein cholesterol.

Discussion

The Spearman's correlation coefficient between D and F was 0.964 in the range of TG < 400 mg/dL. There was a correlation between LogD and LogF ($R^2 = 0.9259$). The average of % Bias was −4.94%. A weak correlation between non-HDL-c and D + 30 was observed ($R^2 = 61\%$).

Friedewald et al.[12] examined the relationship between the LDL-c calculated by their equation and the LDL-c obtained by ultracentrifugation in 448 subjects with normal, type II, and type IV hyperlipidemia (HL). Their equation showed a good relationship with all TG ranges (20 mg/dL-2,502 mg/dL) and showed a better relationship with TG < 400 mg/dL. They reported a correlation coefficient of 0.85 in type IV HL and 0.94 in type IV HL, excluding subjects with TG ≥ 400 mg/dL. In our study, the patients were not biased according to diseases, such as the type of HL. Samples were obtained from patients with various diseases. From our result, in TG < 400 mg/dL, the correlation between D and F was proved by Spearman's correlation coefficient and by average % bias. According to Japanese Association of Medical Technologist,[13] when % bias is 5% or less, it is considered that there is a significant correlation. Although the true value cannot be determined, it was proved that D and F have a correlation. Thus, it could be treated as continuously compared and examined as follow-up data in case of TG < 400 mg/dL. Since it is a premise that the Friedewald equation cannot be used in case of TG ≥ 400 mg/dL, F cannot be handled as follow-up data.

JAS recommends the use of non-HDL-c in patients with TG ≥ 400 mg/dL. Non-HDL-c is defined as TC − HDL-c, which is the summation of VLDL-c, IDL-c, and LDL-c. It represents the risk for all apo-B-containing lipoproteins and is a good marker for atherogenic lipoproteins. In clinical studies, Cui et al.[14] reported that non-HDL-c level is a better predictor of cardiovascular disease mortality than LDL-c. Bittner et al.[15] also reported that non-HDL-c is a strong and independent predictor of non-fatal myocardial infarction and angina pectoris. Non-

HDL-c is considered a predictor of CHD mortality. Moreover, non-HDL-c is simple, convenient, free from dietary influence, and not cost-consuming. Shimano et al.[10] reported that non-HDL-c had a significant correlation with LDL-c, when calculated using the Friedewald equation, in TG < 400 mg/dL. Non-HDL-c was approximately 30 mg/dL higher than the LDL-c concentration.[10] Sugimoto et al.[11] reported that non-HDL-c and D have a good correlation with non-HDL-c = 1.131 × D + 10.88 (r = 0.941), which non-HDL-c value is almost equivalent to D + 30. These are the rationale by which the current non-HDL-c reference value became the management target value of LDL-c + 30 mg/dL. But, in both studies, almost samples were TG < 400 mg/dL. Our study was conducted with TG ≥ 400 mg/dL as JAS guideline recommended, and the correlation between non-HDL-c and D + 30 mg/dL was very weak (R^2 = 61%). Thus, further study is needed to decide the reference value of non-HDL-c in TG ≥ 400 mg/dL.

There were a few samples in which differences between D and F were large, even with TG < 400 mg/dL. Bansal et al.[16] reported a good correlation between D and F in fasting patients at different TC and TG levels ranging from 150 mg/dL to 199 mg/dL and from 101 mg/dL to 200 mg/dL, respectively. A difference was observed at all levels of TC and TG. Tighe et al.[17] investigated the correlation between D and F. D was higher than F at normal or slightly increased TG concentrations. The Friedewald equation assumes that the total TG exists only in VLDL with TG/5 as VLDL-c. However, TG exists not only in VLDL but also in other lipoproteins, such as chyromicrons or VLDL remnants. This would overestimate VLDL-c and lead to underestimation of LDL-c in the Friedewald equation.

We investigated the clinical background that may explain the differences between D and F. In our study, 128 samples over the 95th percentile from the median value of the difference between D and F, in which D was higher than F, had TG levels ranging from 184 mg/dL to 397 mg/dL (data not shown). In these samples, diseases such as diabetes, renal disease, liver disease, pregnancy, thyroid disease, and steroid medication were observed, which may have caused secondary dyslipidemia. There were nine samples lower the 95th percentile from the median value of the difference between D and F, in which D was lower than F. Six out of these nine samples had liver disease, such as liver cancer and liver cirrhosis. These diseases cause lipid metabolic dysfunction, and affect the kinds of lipoproteins, causing increased abnormal lipoproteins. These abnormal lipoproteins may cause discrepancies in D and F; however, we could not confirm the metabolic disorders without electrophoresis examination. Furthermore, liver dysfunction may cause high bilirubin levels, which would interfere and affect direct LDL-c measurement.

This study has several limitations. The samples included were taken in fasting or non-fasting states, which means that our investigation did not fully match Fridewald's requirement. Miida et al.[8] compared LDL-c in 12 homogenous assays with the beta-quantification method (BQ method) in the fasting and non-fasting states in a range of less than 1,000 mg/dL of variety in TG levels. Larger LDL-c differences between the homogenous assay and BQ method were observed in subjects with higher TG levels. We measured the D value using only one direct method (Kyowa Medex). We did not examine other homogenous assays.

Conclusions

In conclusion, a significant correlation was found between D and F. Both D and F could be continuously compared and examined as follow-up data in TG < 400 mg/dL. We proposed to reconsider LDL-c + 30 mg/dL as a reference value of non-HDL-c in TG ≥ 400 mg/dL. Additionally, even in TG < 400 mg/dL, the discrepancies of the value between D and F were associated with severe liver diseases.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Ethical Approval: We obtained approval from the Ethics Committee of Tokyo Women's Medical University (No. 3537) for this study.

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