Original Article

Comparison of LDL-Cholesterol Direct Measurement with the Estimate Using the Friedewald Formula in a Sample of 10,664 Patients

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Objective

To compare direct measurement of LDL-cholesterol (LDL-C) determined by a homogeneous method with LDL-cholesterol estimation determined by the Friedewald formula in a large heterogeneous population.

Methods

The measurements of total cholesterol (TC) and triglycerides (TG) were performed using traditional enzymatic methods. The measurements of HDL-C and LDL-C were performed using direct methods with no precipitation, and the estimation of the LDL-C fraction was calculated using the Friedewald formula.

Results

On linear regression analysis, the 2 methods had extremely significant correlation coefficients ($P < 0.001$). However, the Friedewald formula had a positive bias in regard to the direct method, more pronounced with TC levels > 201 mg/dL. This positive bias also occurred in regard to TG levels ≤ 150 mg/dL. No bias was observed between the methods for TG levels ranging from 151 to 200 mg/dL and from 201 to 300 mg/dL. On the other hand, for TG levels ranging from 301 to 400 mg/dL, this bias of the Friedewald formula became negative.

Conclusion

The Friedewald formula did not have a homogeneous performance for estimating LDL-C levels in samples with different TG levels as compared with that of the direct method, what could launch doubts on patients classification on the risk of developing coronary artery disease.

Key words

cholesterol, LDL-C, direct LDL, Friedewald formula

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Coronary artery disease accounts for the greatest number of deaths of adult individuals worldwide [1]. Several studies have shown the correlation existing between the increased levels of cholesterol in low-density lipoproteins (LDL-C) and the risk of developing that disease [2,3]. The III Brazilian Consensus on Dyslipidemias stratifies the following ranges of LDL-C levels for assessing the risk of developing coronary artery disease: desirable, below 130 mg/dL; borderline, between 130 and 159 mg/dL; and high, above 160 mg/dL [4]. These ranges are very narrow, and the National Cholesterol Education Program (NCEP) established that clinical laboratories should use methodologies for measuring LDL-C levels with a total analytical error < 12%, imprecision < 4%, and inaccuracy < 4% [1]. The reference method for determining LDL-C is β-quantitation [5], which requires ultracentrifugation of the samples, which is not feasible in laboratory routine. Therefore, most laboratories estimate LDL-C levels with the Friedewald formula, based on the concentrations of total cholesterol, the cholesterol present in high-density lipoproteins (HDL-C), and triglycerides [6]. However, the error of determining LDL-C through that estimation comprises the addition of the analytical errors of the 3 parameters used in the calculus, which usually does not meet the NCEP criteria for total error. In addition, the use of that formula has severe limitations and cannot be applied to samples containing triglyceride (TG) levels > 400 mg/dL, to samples with chylomicrons, and to samples of patients with dysbetalipoproteinemia (Fredrickson Type III) [7]. Some authors have demonstrated that that formula should not be used in certain groups of patients, such as patients with diabetes, hepatopathies, or nephropathies, even with triglyceride levels < 400 mg/dL [8].

Recently, several homogeneous methods have been developed by different manufacturers for the direct measurement of LDL-C levels, expecting that the NCEP criteria are met, as well as that the medical community's need to prevent coronary artery disease and myocardial infarction are fulfilled. These methods seem to be better than the previous ones that use selective chemical precipitation or immunoprecipitation, which are laborious and have a significant bias as compared with the reference method [7, 9]. However, mainly due to the costs of the reagents, their use in clinical laboratories has not been largely disseminated, resulting in scarcity of data about the performance and validation of those methods. This study aimed at assessing the performance of a direct homogeneous method for measuring LDL-C and at comparing it with the estimation of LDL-C levels using the Friedewald formula, analyzing a large sample obtained over 2 years of experience with those reagents.

Methods

This study assessed the blood samples of 10,664 patients who sought our laboratory to undergo total cholesterol, LDL-C, HDL-C, and triglyceride measurements from January 2000 to December 2002. Their ages ranged from 14 to 93 years; 5,846 (54.82%) were females, and 4,818 (45.18%) were males. Blood samples were collected after a 12- to 14-hour fast, incubated in a hot-water bath for 15 minutes for coagulation, and centrifuged at 2,000 x g for 5 minutes. The serum was separated and the assays were performed on the same day of sample collection.

The measurements of the triglycerides and total cholesterol were performed with the reagents Triglycerides FS (DiaSys Diagnostic Systems GmbH & Co. KG, Holzheim, Germany), and Cholesterol (BioSystems S.A., Barcelona, Spain), respectively, according to the specifications of the manufacturers, in a Spectrum CCX II device (Abbott Diagnostics, Abbott Park, IL, USA). The tests were calibrated with the CCX Multicalibrator Set (Abbott), with curves of 3 points.

The LDL-C measurement with the homogeneous method was performed with the reagent LDL-C Select FS (DiaSys), according to the specifications of the manufacturer. The method is based on the selective protection of LDL-C with the addition of reagent 1 [Good's buffer, pH 6.8, 22 mmol/L, cholesterol esterase ≥ 2 kU/L, cholesterol oxidase ≥ 2 kU/L, N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (H-DAOS) 0.43 mmol/L, catalase ≥ 400 kU/L, final concentrations]. The cholesterol of the other lipoproteins is processed by cholesterol oxidase, and the hydrogen peroxide formed is broken down by catalase. After 5 minutes, with the addition of reagent 2 (Good's buffer, pH 7.0, 22 mmol/L, 4-aminoantipyrine 0.68 mmol/L, peroxidase ≥ 3 kU/L, final concentrations), LDL-C is released for enzymatic processing and development by the Trinder reaction. All reagents are stable fluids. According to the manufacturer, no interference occurs with triglyceride levels up to 1,000 mg/dL, bilirubin up to 50 mg/dL, hemoglobin up to 500 mg/dL, or ascorbic acid up to 50 mg/dL. The tests were performed using special programming in a Spectrum CCX II device (Abbott) calibrated with TruCal (DiaSys).

For samples with triglyceride levels < 400 mg/dL, the LDL-C level was estimated using the Friedewald formula: LDL-C = TC – HDL-C – (TG/5) [6].

The HDL-C measurement was performed using a homogeneous method without precipitation with the HDL-C Immuno FS reagent (DiaSys). The method is based on the formation of immunocomplexes of LDL and VLDL lipoproteins and chylomicrons with human anti-β lipoprotein antibodies after the addition of reagent 1 (Good's buffer, pH 7.0, 26 mmol/L, 4-aminoantipyrine 0.60 mmol/L, peroxidase 1,600 U/L, ascorbate oxidase 1,800 U/L, final concentrations, and sheep antibodies anti human-β lipoproteins). Then, enzymatic processing of HDL-C occurred with the addition, after 5 minutes, of reagent 2 [Good's buffer, pH 7.0, 26 mmol/L, cholesterol esterase 800 U/L, cholesterol oxidase 4,000 U/L, sodium N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoroaniline 0.16 mmol/L]. All reagents are stable fluids. According to the manufacturer, no interference occurs with triglyceride levels up to 1,200 mg/dL, bilirubin up to 40 mg/dL, hemoglobin up to 500 mg/dL, or ascorbic acid up to 50 mg/dL. The tests were performed using special programming in a Spectrum CCX II device (Abbott) calibrated with TruCal (DiaSys).

The coefficient of variation of the tests performed was determined by analysis of the results obtained during 20 consecutive days using Accumark control serum aliquots (Sigma Diagnostics, St. Louis, MO, USA), 111K6403 lot.

The comparison between the LDL-C measurement methods, homogeneous and estimation through the Friedewald formula, was performed according to the Passing and Bablok method [10], using analysis of correlation expressed by the equation $y = bx + a$, where *b* is the gradient of the line and represents the proportional error, and *a* is the intersection in the *y* axis and represents the constant error. To improve the comparison between the methods, the samples were stratified according to total cholesterol levels (70-150 mg/dL, 151-200 mg/dL, 201-250 mg/dL, and 251-550 mg/dL) and to triglyceride levels (≤ 150 mg/dL, 151-200 mg/dL, 201-300 mg/dL, and 301-400 mg/dL).

The statistical analysis of the results was performed with the aid of GraphPad InStat and GraphPad Prism software (San Diego, CA, USA). The significance level adopted was $P < 0.05$. Mean, standard deviation, and coefficient of variation were calculated with the aid of Excel software (Microsoft).

Results

The coefficient of variation of the LDL-C measurement using the homogeneous method was 4%. The coefficients of variation of the total cholesterol, triglyceride, and HDL-C measurements were 3%, 4%, and 3%, respectively. In regard to LDL-C measurements, the National Cholesterol Education Program (NCEP) recommends imprecision ≤ 4% [1]. The measurements performed in our laboratory met that criterion.

The concentrations of total cholesterol and lipoproteins obtained in this study in regard to the different triglyceride levels are shown in table I. The concentrations of triglycerides and lipoproteins in regard to the different levels of total cholesterol are shown in table II.

The comparison of the methods [homogeneous LDL-C (*x*) versus calculated LDL-C (*y*)] resulted in the following regression equations: $y = 0.6905\,x + 27.9$, for total cholesterol levels between 70 and 150 mg/dL; $y = 0.6387\,x + 44.8$, for total cholesterol levels between 151 and 200 mg/dL; $y = 0.6039\,x + 63.7$, for total cholesterol levels between 201 and 250 mg/dL; and $y = 0.7256\,x + 60.7$, for total cholesterol levels > 250 mg/dL. According to the total cholesterol levels, the correlation coefficients between the methods of 0.6105, 0.6160, 0.6735, and 0.7822, respectively, were extremely significant (< 0.001). However, the LDL-C estimate determined with the Friedewald formula tends towards producing slightly greater results as compared with those from LDL-C measurements using the homogeneous method. This bias practically does not occur with cholesterol levels up to 150 mg/dL, in which a mean difference of 7 ± 12.1 mg/dL (11.5% ± 16.1%) was observed, with a proportional error of –30.9% [(gradient of the line - 1) x 100), and a constant error of +27.9 mg/dL. However, with cholesterol levels between 151 and 200 mg/dL, the constant error increased to +44.8 mg/dL, and the proportional error was -36%, which resulted in a mean deviation of 8 ± 14.5 mg/dL (9.5% ± 13.3%). In the same way, with cholesterol levels between 201 and 250 mg/dL and > 250 mg/dL, the constant error increased to +63.7 mg/dL and +60.7 mg/dL, respectively, with proportional errors of –40% and –27%, respectively. This resulted in mean deviations of 10 ± 15.3 mg/dL (8.3% ± 10.5%), and 10 ± 20.4 mg/

Table I - Summary of the measurements of total cholesterol, LDL-C (direct), LDL-C (Friedewald), and HDL-C according to triglyceride levels, presented as mean ± standard deviation (lowest level found – greatest level found)

Triglycerides	Total cholesterol	LDL-C (direct)	LDL-C (Friedewald)*	HDL-C
≤ 150 mg/dL	211 ± 43 (73-452)	126 ± 37 (24-307)	140 ± 39 (28-327)	51 ± 12 (12-103)
151-200 mg/dL	234 ± 42 (106-475)	146 ± 39 (39-321)	153 ± 40 (41-376)	46 ± 10 (18-103)
201-300 mg/dL	241 ± 45 (130-455)	152 ± 42 (56-332)	150 ± 43 (37-325)	40 ± 10 (19-103)
301-400 mg/dL	249 ± 47 (87-393)	157 ± 45 (40-299)	141 ± 45 (27-278)	39 ± 10 (14-79)
> 400 mg/dL	265 ± 54 (152-523)	163 ± 57 (57-423)	-	37 ± 9 (16-71)

* Applied to samples with triglyceride levels ≤ 400 mg/dL.

Table II - Summary of the measurements of triglycerides, LDL-C (direct), LDL-C (Friedewald), and HDL-C according to total cholesterol levels, presented as mean ± standard deviation (lowest level found – greatest level found)

Total cholesterol	Triglycerides	LDL-C (direct)	LDL-C (Friedewald)*	HDL-C
≤ 150 mg/dL	89 ± 53 (20-374)	68 ± 13 (24-105)	75 ± 14 (27-107)	42 ± 10 (12-74)
151-200 mg/dL	119 ± 61 (18-399)	101 ± 16 (47-155)	110 ± 16 (46-156)	46 ± 11 (14-93)
201-250 mg/dL	146 ± 69 (21-398)	136 ± 20 (73-200)	146 ± 18 (69-198)	49 ± 12 (16-120)
> 250 mg/dL	176 ± 76 (58-400)	185 ± 32 (47-332)	195 ± 30 (115-376)	52 ± 13 (21-130)

* Applied to samples with triglyceride levels ≤ 400 mg/dL.

dL (6.5% ± 10.5%), respectively. The results of these comparisons are summarized in table III and figure 1.

The comparison of the methods [homogeneous LDL-C (x) versus calculated LDL-C (y)] resulted in the following regression equations: $y = 0.9746\ x + 17.6$, for triglyceride levels ≤ 150 mg/dL; $y = 0.9593\ x + 12.6$, for triglyceride levels between 151 and 200 mg/dL; $y = 0.9459\ x + 6.7$, for triglyceride levels between 201 and 300 mg/dL; and $y = 0.8999\ x - 0.1$, for triglyceride levels between 301 and 400 mg/dL. In those different triglyceride levels, the correlation coefficients between the methods of 0.9426, 0.9332, 0.9345, and 0.9072, respectively, were extremely significant (< 0.001). However, the LDL-C estimate using the Friedewald formula also tends towards producing greater results as compared with those obtained in LDL-C measurements through the homogeneous method for triglyceride levels up to 150 mg/dL, in which a mean difference of 14 ± 13 mg/dL (12.8% ± 9.2%) was found, with a constant error of +17.6 mg/dL, and a proportional error of –3%. With triglyceride levels between 151 and 200 mg/dL, this deviation tends towards decreasing, with a mean difference of 7 ± 14.5 mg/dL (5.3% ± 9.5%), a proportional error of -4%, and a constant error of +12.6 mg/dL. With triglyceride levels between 201 and 300 mg/dL, this deviation almost does not exist: 2 ± 15.3 mg/dL (0.8% ± 10%), with a proportional error of -5% and a constant error of +6.7 mg/dL. On the other hand, with triglyceride levels between 301 and 400 mg/dL, the LDL-C estimate with the Friedewald formula tends towards producing lower values, with a mean difference of -16 ± 19.4 mg/dL (-10.3% ± 12.4%), as compared with the LDL-C measurement using the homogeneous method, in which a proportional error of -10% and a constant error of -0.1 mg/dL are observed (tab. III and fig.2).

Discussion

This study aimed at assessing the performance of a homogeneous method for direct LDL-C measurement, as compared with the LDL-C estimate by using the Friedewald formula. Despite the technological innovations, the Friedewald formula continues to be used in many laboratories, and its application has been recommended by the III Brazilian Consensus on Dyslipidemias for samples with triglyceride levels up to 400 mg/dL. However, as already reported by other authors [9,11-17] and ourselves, the homogeneous methods and the Friedewald formula are not capable of providing identical results. This conclusion is also evident when the results of the samples of this study are analyzed according to their respective levels of total cholesterol and triglycerides.

Assessing the results according to the different levels of total cholesterol, the LDL-C estimate through the Friedewald formula was observed to have an extremely significant correlation ($P < 0.001$), as compared with the direct method (tab. II). However, the correlation coefficients between the 2 methods are not extremely close (0.6105 to 0.7822) (tab. III). In fact, the Friedewald formula has a positive deviation or bias in regard to the direct method. This bias is neither very pronounced with total cholesterol levels between 70 and 150 mg/dL (mean deviation of 7 ± 21 mg/dL), nor with total cholesterol levels between 151 and 200 mg/dL (8 ± 14.5 mg/dL). But, from total cholesterol levels between 201 and 250 mg/dL onwards, this deviation tends towards increasing, with a mean of 10 ± 15.3 mg/dL. Similarly, with total cholesterol levels > 250 mg/dL, a positive bias of 10 ± 20.4 mg/dL occurs. Therefore, if a patient had an LDL-C level of 125 mg/dL on the direct method, the result by using the estimate of the Friedewald formula could be 139 mg/dL (fig. 1B), considering the linear regression equation obtained for total cholesterol levels between 201 and 250 mg/dL ($y = 0.6039\ x + 63.7$). Therefore, although with no statistical significance, theoretically, LDL-C levels of a part of the population could pass from desirable values (< 130 mg/dL) to borderline values (130-159 mg/dL), which are subject to dietary control and even treatment with statins. Similarly, patients classified as within borderline values through the direct method could pass into the high value range (160-189 mg/dL) through estimate calculation by using the Friedewald formula (fig. 1C).

On the other hand, when assessing the results according to the different levels of triglycerides, a pattern of inversion of that bias is observed (tab. I), although the 2 methods have excellent correlation coefficients (0.9072 to 0.9426) (tab. III). With triglyceride levels up to 150 mg/dL, a mean positive bias of 14 ± 13 mg/dL is observed for the Friedewald formula. Therefore, theoretically, even without statistical significance, a patient with triglycerides < 150 mg/dL and an LDL-C level of 125 mg/dL, by using the direct method, could have an LDL-C level estimated through the Friedewald formula of 139 mg/dL (fig. 2A), considering the linear regression equation for that triglyceride range ($y = 0.9746x + 17.6$). That hypothetical patient would pass from a desirable LDL-C value to a borderline value. The same would happen to a patient with an LDL-C level of 150 mg/dL measured by the direct method, who would pass from the range of borderline values to that of high values (164 mg/dL) according to the estimate through the formula.

Table III - Summary of the comparison of the direct method for LDL-C measurement with the LDL-C estimate by use of the Friedewald formula, according to the triglyceride and total cholesterol levels, analyzed through linear regression [a]

		Pearson correlation coefficient	Gradient (95% CI)[b]	Y Intersection (95% CI) mg/dL	$S_{y.x}$ mg/dL [c]
Triglycerides					
≤ 150 mg/dL	520	0.9426	0.9746 (0.9662 to 0.9830)	17.6 (16.5 to 18.7)	12.9
151-200 mg/dL	897	0.9332	0.9593 (0.9427 to 0.9760)	12.6 (10.1 to 15.2)	14.4
201-300 mg/dL	458	0.9345	0.9459 (0.9274 to 0.9644)	6.7 (3.7 to 9.6)	15.2
301-400 mg/dL	48	0.9072	0.8999 (0.8612 to 0.9387)	- 0.1 (-6.4 to 6.2)	18.9
Total cholesterol					
≤ 150 mg/dL	72	0.6105	0.6905 (0.6093 to 0.7717)	27.9 (22.3 to 33.6)	11.5
151-200 mg/dL	3035	0.6160	0.6387 (0.6097 to 0.6678)	44.8 (41.8 to 47.8)	13.3
201-250 mg/dL	4376	0.6735	0.6039 (0.5843 to 0.6236)	63.7 (61.0 to 66.4)	13.1
> 250 mg/dL	462	0.7822	0.7256 (0.7027 to 0.7484)	60.7 (56.5 to 65.1)	18.4

[a] In the form of $y = ax + b$, where y = calculated LDL (Friedewald); x = direct LDL; a = gradient of the line; b = y intersection; [b] CI: confidence interval; [c] standard deviation of the residues y.x

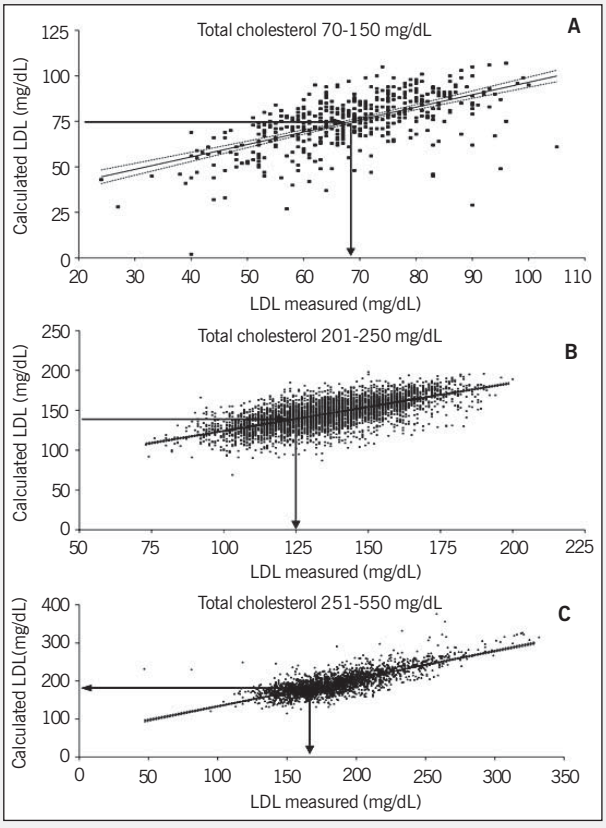

Fig. 1 - Graphic representation of the comparison of LDL-C measurement by use of the homogeneous method with LDL estimate by use of the Friedewald formula, in the following total cholesterol ranges: 70-150 mg/dL (A); 201-250 mg/dL (B); and > 251-550 mg/dL (C).

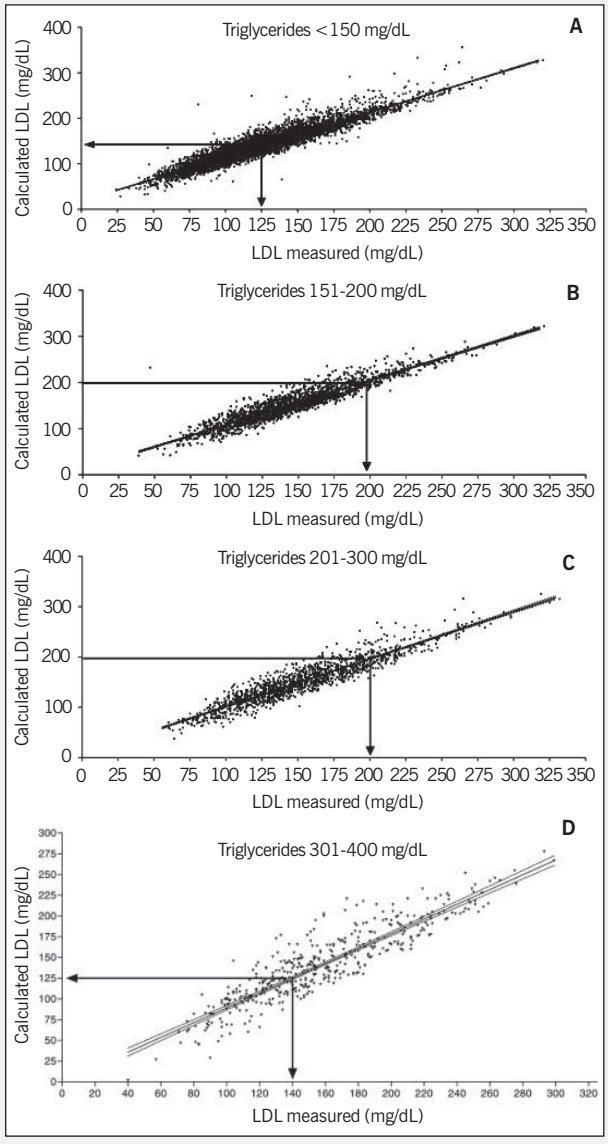

Fig. 2 - Graphic representation of the comparison of LDL-C measurement by use of the homogeneous method with LDL estimate by use of the Friedewald formula, in the following triglyceride ranges: up to 150 mg/dL (A); 151-200 mg/dL (B); 201-300 mg/dL (C); and 301-400 mg/dL (D).

Several authors reported lower results obtained through the direct methods of LDL-C measurement as compared with those obtained through β-quantitation after ultracentrifugation [11,12]. Others, however, reported that bias with a certain reagent, and a perfect correlation with another reagent, although both used detergents, but with different principles [13]. The reagents used in this study follow a principle of detergent protection similar to the method used in the study cited, which reported no bias, as compared with the reference method. Some authors have also not found that variation in bias in regard to different levels of triglycerides with a method that also uses specific tensoactive agents [12], differently from most direct methods [9, 14-18], which may be due to a difference in the components of the reagents.

In our study, for triglyceride levels between 151 and 200 mg/dL, that bias decreased to 7 ± 14.5 mg/dL, and, for triglyceride levels between 201 and 300 mg/dL, that bias almost did not exist (2 ± 15.3 mg/dL).

On the other hand, in the samples with triglyceride levels between 301 and 400 mg/dL, that bias of the Friedewald formula became negative, with a mean deviation of -16 ± 19.4 mg/dL. This way, as can be demonstrated with our results, a patient with triglyceride levels between 301 and 400 mg/dL and an LDL-C level measured through the direct method of 140 mg/dL could theoretically have an LDL-C level estimated through the Friedewald formula of 126 mg/dL (fig. 2D), considering the linear regression equation for that range of triglyceride levels ($y = 0.8999x - 0.1$), although no statistical significance was observed. The patient would pass from a borderline LDL-C value to a desirable LDL-C value. The same would happen to a patient with an LDL-C level of 170 mg/dL measured through the direct method, who would pass from the range of high values to the range of borderline values (153 mg/dL).

According to other authors [13,19], one explanation for these higher LDL-C levels obtained through the direct method as compared with those obtained through the Friedewald formula could be the difference in the triglyceride/cholesterol ratio in the VLDL particles in patients with types IIb, III, and IV Fredrickson dyslipidemias. Triglyceride-rich VLDL particles could induce a negative bias in the direct methods [13]. Cholesterol-rich VLDL particles could induce a positive bias [19]. However, this would not explain the positive bias of the direct method in patients with high triglyceride levels in our study.

Another explanation would be the possibility of measuring the cholesterol present in the particles of intermediate-density lipoproteins (IDL), such as LDL-C, in patients with type III Fredrickson dyslipidemia through some direct methods [13]. This could be happening with the method in question, but that hypothesis cannot explain the tendency in all patients with triglyceride levels between 301 and 400 mg/dL, shown in our study. In fact, as reported by those authors, those patients with type III dyslipidemia would tend to be erroneously classified as type IIb using the direct method for measuring LDL-C, overestimating the LDL-C fraction. However, the estimate through the Friedewald formula does not help in the correct classification, overestimating the VLDL fraction (triglycerides/5), because it cannot indicate the presence of IDLs, which could also be suspected only because of the high triglyceride levels. In the specific case of patients with type III dyslipidemia, good communication between the clinician and the laboratory is an excellent contribution to the diagnosis, because the latter would be able to determine whether the method used may overestimate the LDL-C fraction, in the case of the direct method, or the VLDL fraction, in the case of estimate through the Friedewald formula.

For LDL-C estimate, the Friedewald formula standardizes the value of the VLDL fraction as the triglyceride level divided by 5. However, the particles found in patients with hypertriglyceridemia (types IIb, III, IV, and V) are usually a heterogeneous mixture of remaining chylomicrons, VLDL, and remaining VLDL (IDLs). As is already known, the triglyceride/cholesterol ratio varies a lot within that range of particles. In fact, according to our results, the LDL-C estimate through the Friedewald formula has good performance in samples with triglyceride levels between 151 and 300 mg/dL, as compared with the direct method. However, in the extremes of that range, in the samples with triglyceride levels < 150 mg/dL and between 301 and 400 mg/dL, the formula does not have good performance. In fact, according to some authors, the Friedewald formula may erroneously classify up to 25% of the patients with triglyceride levels between 301 and 400 mg/dL [13]. That percentage may be even higher, depending on the method used for measuring HDL-C levels.

Thus, patients with triglyceride levels < 150 mg/dL and desirable LDL-C levels measured through the direct method could be being treated based on an LDL-C level estimated through the Friedewald formula. On the other hand, patients with triglyceride levels between 301 and 400 mg/dL and borderline or high LDL-C levels measured through the direct method would not be receiving the appropriate treatment with the results obtained through the use of the Friedewald formula. Obviously, the limits of LDL-C levels for risk classification for coronary artery disease are very narrow, but as those are the values recommended by the III Brazilian Consensus on Dyslipidemias [4], the clinical laboratory should strive to perform the measurement of that fraction with the best possible diagnostic performance, ie, with the lowest coefficient of variation, and with the best likelihood of correctly classifying the patients for assessing the risk for coronary artery disease.

This study, analyzing a sample with more than 10,000 patients, demonstrated that the direct method used for measuring LDL-C has very good performance, with good reproducibility and a coefficient of variation within the requirements of the NCEP, which is hardly obtained with the Friedewald formula. It is worth noting, however, that although one of the objectives was to discuss that the 2 methods do not have identical results, it is still a consensus that the Friedewald formula may be used in patients with triglyceride levels up to 400 mg/dL, who have neither chylomicrons, nor IDLs (type III of the Fredrickson classification) [4,20]. In fact, many laboratories still continue to estimate LDL-C levels by using that formula, because of the costs of the reagents for the existing direct LDL-C measuring methods. With a decrease in those costs and a better assessment of the performance of the reagents, the direct methods tend to be more widely used in laboratories, providing a better classification of the patients, with more reliable LDL-C level results, according to the NCEP criteria. Certain populations would benefit extremely by the use of the direct methods, such as diabetic patients, who are naturally prone to developing coronary artery disease, and whose LDL-C levels are not correctly estimated with the Friedewald formula [8].

In the future, new methods may be implemented for determining LDL-C levels, such as one recently described, which is reagent-free and based on infrared spectrophotometric absorption of lipoproteins [21]. That method had excellent performance, and its assessment may shed light on the debate about the introduction of new methods for LDL-C measurement.

Recently, some authors reported that 2 direct methods for LDL-C measurement did not have good retrieval of the small, dense LDL subtype (sdLDL), previously separated by ultracentrifugation [22]. The determination of that LDL subtype levels has been implicated as a more sensitive factor for assessing the risk of developing coronary artery disease than the determination of total LDL-C has. Methods for the routine sdLDL measurement are still being developed, but, in the near future, the laboratories may also be including that parameter in the lipid and lipoprotein profile available for clinicians [23].

Similarly, the direct method for HDL-C measurement had ex-

cellent performance, with a very low coefficient of variation. In our experience, that method has excellent performance in laboratory routine, is easily automated, and does not suffer from interference, as occurs in precipitation methods [1], which cause additional error in LDL-C estimated with the Friedewald formula. Other authors [24] reported that, in comparison with the reference method, the direct method does not provide lower HDL-C levels, as occurs in precipitation methods.

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