Friedewald Formula Significantly Underestimates LDL Cholesterol Compared to Preparative Ultracentrifugation below 70 mg/dL leading to Overestimation of the LDL Cholesterol Reduction for New drugs in Development.

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Abstract

Background: Calculated LDL-C by Friedewald formula has been the basis for clinical and regulatory decision making for 40 years. The validity at low LDL-C has recently been questioned as clinical guidelines and new therapeutic agents reduce LDL-C levels to below levels originally validated by Friedewald. We compare LDL-C by Friedewald and the 'gold standard', preparative ultracentrifugation (PUC) in 68,751 samples, with 23,488 ≤70 mg/dL and 16,785 ≤50 mg/dL.

Methods: Serum or plasma samples from patients in a specialized lipid clinic and clinical trials over the last 6 years analyzed by Friedewald and PUC were compared, in a central laboratory CDC-NHLBI Part 3 Standardized for lipid measurements. Clinically important cut-points of 100, 70, 50 and 25 mg/dL and within each cut-point by triglyceride (TG) levels of ≤100, 101≤200, 201≤300, 301≤400 mg/dL assessed.

Results: For LDL-C >100 mg/dL there was minimal difference between methods and triglyceride levels had minimal impact. Differences became apparent between 100 and 70 mg/dL and calculated LDL-C at 51-70, 26-50 and ≤25 mg/dL averaged 5.5, 8.2 and 27.7% lower than by PUC, respectively. Friedewald further underestimated PUC LDL-C for each 100 mg/dL TG increase above 100 mg/dL by 33, 57 and 68% when LDL-C was ≤25 mg/dL.

Conclusion: Friedewald accurately measures LDL-C >100 mg/dL, the usual entry for clinical trials, but significantly underestimates LDL-C <70 mg/dL and more so <50 mg/dL. This has clinical implications that can result in high risk CAD patients being undertreated by in reality not achieving a goal of <70 mg/dL or having statin therapy reduced when calculated LDL-C is <40 mg/dL. There are consequences for new drug development and comparison of efficacy with older drugs as Friedewald overestimates the apparent reduction with treatment at lower LDL-C. This overestimation increases at even lower LDL-C and is compounded by even moderate TG elevations. These factors combined may have major implications for new CVD outcome trials.

Introduction

▪ Since the first NCEP-ATP Guideline, LDL-C has been the focus and basis for therapeutic decisions.

• Recent AHA/ACC guidelines[1] minimize LDL-C treatment goals, but guidelines throughout the world continue to recommend therapeutic targets s.

• Given the substantial controversy and debate since the release of the AHA/ACC guidelines it is unclear how successfully they will influence physician practice regarding the use of LDL-C treatment targets.[2,3]

• Irrespective of the AHA/ACC guideline acceptance they do continue to rely on specific LDL-C cut-points and percent LDL-C reductions for instituting drug therapy and achieving therapeutic goals. e.g, patients with CAD or at very high risk for CAD are recommended to start statin therapy if LDL-C is >70 mg/dl.[1] There is also the recommendation that patients achieving a LDL-C below 40 mg/dL on statins should consider dose reduction. Thus accurate measurement of LDL-C remains a vital component of our decision making in terms of even the new 'non-target' guidelines.

•The most commonly used method is to calculate LDL-C using the Friedewald formula, widely accepted as an accurate and cost-effective alternative to the reference method, preparative ultracentrifugation (PUC), for routine clinical purposes in patients with TG <400 mg/dL (<4.52 mmol/L).[4]

• The Friedewald formula has also been used extensively, and reliably, for 30 years in developing lipid-modifying drugs. However, highly efficacious statins, add on therapy with second-line agents such as ezetimibe, and CVD outcome trials such as JUPITER, where baseline LDL-C levels were close to 100 mg/dL, has resulted in achievement of LDL-C levels below the lower ranges included in the original validation of the formula.[5]

• In 2001 Scharnagl et al first reported that the Friedewald formula underestimated LDL-C in patients with low LDL-C undergoing apheresis.[6] Data from a number recent studies from either patients undergoing lipid measurement in routine clinical practice or participating in clinical trials have provided additional data that calculated LDL-C values may not be accurate when LDL-C decreases below 70 mg/dL (1.8 mmol/L).[7,8]

• Preparative ultracentrifugation (PUC) is recognized as the most accurate, and is accepted as the 'gold standard' method for measurement of LDL-C.[9].

• We report the results of LDL-C measured by both Friedewald and preparative ultracentrifugation in 68,751 patient samples, including 16,785 below 50 mg/dL. We also report the absolute and percent reductions in LDL-C for 10,190 patients where pre and post treatment LDL-C allowed for the comparison by both methods.

Methodology

Samples

Serum or plasma samples collected after an overnight fast (water only) and analyzed for total cholesterol, triglyceride, HDL-C and LDL-C by PUC in the laboratory since 2006 were evaluated and those with triglyceride ≤400 mg/dL and where all analysis were available were to qualify for calculation of LDL-C by the Friedewald formula were included, resulting in total of 68,751 comparisons.

The samples were from either patients in a specialized lipid clinic or participants in clinical trials, and included pediatric subject samples. All samples were received de-identified as to demographic information.

Samples from subjects with repeated visits allowed for the assessment of visit to visit changes in LDL-C from their initial sample with a total of 10,190 pairs available by both Friedewald and PUC for comparison.

Methodology (Continued)

Analytical methods

•Total cholesterol (TC), the cholesterol content of isolated fractions, and TG were measured in the central laboratory (Medpace Reference Laboratories, Cincinnati, USA and Leuven, Belgium), which maintained CDC-NHLBI Lipid Standardization Program Part III throughout the period (Participant numbers LSP-395 and INT-406).[9]

•Analysis of cholesterol and triglycerides was by enzymatic methods on a Beckman Coulter AU2700/AU5400 automatic analyzer with in-house developed serum calibrators directly traceable to CDC-NHLBI reference procedures.[9]

•PUC was performed using the method outlined in the Lipid Research Clinics methods manual.[10] Serum or plasma was overlayed with normal saline (density 1.006 g/mL) and centrifuged (Beckman Ultracentrifuge Model # L-90K and rotor, Type 50.4) at 40,000 rpm for 18–22 hours at 10° C to separate very low-density lipoprotein (VLDL) in the supernatant ('top' fraction) from LDL and HDL in the infranatant or 'bottom' fraction. The cholesterol concentration of the infranatant was measured. All apolipoprotein B-containing lipoproteins, VLDL, LDL and Lp(a), were precipitated from serum using 50 kDa dextran sulfate with magnesium ions ($MgCl_2$)[11], and the cholesterol in the remaining HDL fraction was measured. The HDL-C concentration was subtracted from the infranatant cholesterol to provide the PUC LDL-C value. Very low density lipoprotein cholesterol (VLDL-C) was calculated by subtracting the 'bottom' fraction cholesterol from total cholesterol. The ratio of cholesterol in VLDL to total triglyceride (TG) was calculated by VLDL-C/TG. Calculated LDL-C was derived from the Friedewald formula[9] where: LDL-C = TC – (HDL-C + TG/5) [for mmol/L, LDL-C = TC – HDL-C – (TG/2.2)].

Statistical methods

•Summary statistics, mean (standard deviation [SD]) values for continuous variables, and numbers of patients and percentages for categorical variables were calculated on measured lipid parameters (e.g., total cholesterol, HDL-C, triglycerides, LDL-C by PUC) and calculated lipid parameters (e.g., LDL-C by Friedewald, VLDL-C, VLDL-C/TG ratio).

•Subgroup analyses based on the difference between the calculated and PUC LDL-C for each sample were performed based on calculated LDL-C and triglyceride levels at selected cut points. Similar analysis was done for VLDL-C/TG ratio.

•Differences between Friedewald and PUC in absolute and percentage reductions were assessed from initial sample to last available sample for that subject. A linear regression model was performed with percent change from initial sample in LDL-C by Friedewald as the dependent variable and percent change from initial sample in LDL-C by PUC as the independent variable. Reductions were also assessed by the achieved calculated LDL-C value.

Results

• Overall results for the 68,751 samples are shown in Table 1. LDL-C ranged from 1 to 713 mg/dL by PUC and 0 to 723 mg/dL by Friedewald with an overall mean (±SD) difference of 4.9±15.4%. TG ranged from 14 to 400 mg/dL.

▪ Assessment based on selected calculated LDL-C cut-points (Table 2) resulted in 23,488 results ≤70 mg/dL, 16,785 ≤50 mg/dL and 7092 ≤25 mg/dL.

▪ The Friedewald formula was very accurate compared to PUC between 100 and 200 mg/dL (mean of 137.9 mg/dL for both and mean difference of 0.3%), and slightly higher when over 200 mg/dL (1.9% difference). However as values decreased below 100 mg/dL Friedewald underestimated LDL-C compared to PUC by 2.6% between 100 and 71 mg/dL, 5.5% between 70 and 51 mg/dL, 8.2% between 50 and 26 mg/dL and 27.7% at 25 mg/dL or below. Within each LDL-C cut-point the difference between Friedewald and PUC increases for every 100 mg/dL rise in triglyceride, especially at LDL-C below 50 and 25 mg/dL. Analysis within in each LDL-C cut-point by each 100 mg/dL increase of triglycerides (i.e. ≤100, 101≤200, 201≤300, 301≤400) is shown in Tables 3 and 4 which show mean percent and mean absolute differences between Friedewald and PUC respectively.

Table 1. **Mean (SD) values for all lipid parameters**

Lipid Parameter (units)	N	Mean	SD	Min	Max
Total Cholesterol (mg/dL)	68751	176.5	62.15	34	796
HDL-C (mg/dL)	68751	50.9	16.27	5	210
Triglycerides (mg/dL)	68751	144.4	78.55	14	400
Calculated LDL-C by Friedewald (mg/dL)	68751	96.8	58.79	0	723
PUC LDL-C (mg/dL)	68751	98.8	56.87	1	713
Percent Difference Friedewald[a] (%)	68751	-4.9	15.40	-100	400
VLDL-C[b] (mg/dL)	68751	26.8	17.01	1	278
VLDL-C/Triglycerides	68751	0.185	0.0564	0.011	0.964

[a] Percent difference = 100*(calc LDL-C Friedewald – PUC LDL-C)/ PUC LDL-C [b] VLDL-C = Total cholesterol – HDL-C – PUC LDL-C

• The impact of under-estimation of LDL-C by Friedewald at lower LDL-C levels on assessment of LDL-C reduction is shown in Table 5. The lower the achieved LDL-C level due to treatment the greater the over-estimation of mean percent reduction; when the achieved LDL-C is ≤50 mg/dL the difference in LDL-C reduction was 3.9% (64.3% by Friedewald compared to 60.4% by PUC) and 6.8% when LDL-C was <25 mg/dL (81.2% by Friedewald versus 74.4% by PUC).

• The analysis at a LDL-C of 40 mg/dL by Friedewald (Figure 1), the point suggested by the new AHA/ACC guidelines to reduce statin therapy indicates that compared to PUC nearly 20% of patients will be incorrectly classified.

Results (Continued)

Table 2. **Summary Statistics of Calculated LDL-C (Friedewald) and PUC LDL-C by selected LDL-C cut-points**

LDL-C by Friedewald (mg/dL)		Measured	Calculated		
		PUC LDL-C (mg/dL)	Friedewald LDL-C (mg/dL)	Percent Difference[a] (%)	
	N	Mean (SD)	Mean (SD)	Mean (SD)	P-value
≤25	7092	21.8 (7.93)	15.7 (7.01)	-27.7 (30.17)	<0.0001
26-50	9693	40.5 (9.37)	36.5 (6.88)	-8.2 (13.57)	<0.0001
51-70	6703	65.3 (8.74)	61.1 (5.72)	-5.5 (10.68)	<0.0001
71-100	14019	88.8 (10.89)	85.9 (8.58)	-2.6 (8.74)	<0.0001
101-200	29136	137.9 (25.15)	137.9 (25.19)	0.3 (6.84)	<0.0001
>200	2108	260.0 (90.26)	263.7 (89.54)	1.9 (7.65)	<0.0001
≤50	16785	32.6 (12.77)	27.7 (12.39)	-16.4 (24.16)	<0.0001
≤70	23488	41.9 (18.90)	37.2 (18.63)	-13.3 (21.77)	<0.0001
≤100	37507	59.5 (27.97)	55.4 (28.29)	-9.3 (18.77)	<0.0001

[a] Percent difference = 100*(Calculated LDL-C by Friedewald – PUC LDL-C)/PUC LDL-C

* P-values are from a one sample t-test performed on percent differences.

Table 3. **Percent Difference* between Calculated LDL-C by Friedewald and PUC LDL-C by selected LDL-C and Triglyceride cut-points**

LDL-C by Friedewald (mg/dL)	Triglyceride Level (mg/dL)									
	≤100		101 - 200		201 - 300		301 - 400		Overall	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
≤25	3038	-13.2 (23.89)	3205	-33.0 (27.04)	650	-57.0 (30.01)	199	-67.5 (31.20)	7092	-27.7 (30.17)
26-50	4868	-3.2 (10.78)	3728	-10.5 (12.92)	772	-21.2 (13.84)	325	-26.1 (15.30)	9693	-8.2 (13.57)
51-70	2675	-1.7 (7.62)	2508	-5.7 (9.42)	1056	-11.0 (11.65)	464	-13.7 (17.49)	6703	-5.5 (10.68)
71-100	5010	-0.7 (6.64)	5875	-2.9 (7.57)	2208	-5.1 (11.35)	926	-5.3 (14.31)	14019	-2.6 (8.74)
101-200	8312	-0.2 (4.25)	13840	-0.2 (5.33)	4969	0.7 (9.19)	2015	3.8 (13.55)	29136	0.3 (6.84)
>200	551	0.5 (2.61)	932	0.8 (4.09)	438	3.1 (6.29)	187	8.7 (20.10)	2108	1.9 (7.65)
Overall	24454	-2.7 (11.52)	30088	-5.9 (15.15)	10093	-7.1 (19.27)	4116	-5.8 (23.07)	68751	-4.9 (15.40)

Note: Percent difference = 100*(calculated LDL-C – PUC LDL-C)/PUC LDL-C

* All P<0.001. P-values are from a one sample t-test performed on percent difference.

Table 4. **Absolute Differences (mg/dL) between Calculated LDL-C by Friedewald and PUC LDL-C by selected LDL-C and Triglyceride cut-points**

LDL-C by Friedewald (mg/dL)	Triglyceride Level (mg/dL)									
	≤100		101 - 200		201 - 300		301 - 400		Overall	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
≤25	3038	-2.8 (3.39)*	3205	-6.8 (4.48)*	650	-13.4 (6.11)*	199	-21.1 (9.52)*	7092	-6.1 (6.01)*
26-50	4868	-1.6 (3.86)*	3728	-4.8 (5.17)*	772	-11.1 (7.13)*	325	-15.6 (9.55)*	9693	-4.1 (6.05)*
51-70	2675	-1.4 (4.39)*	2508	-4.2 (5.95)*	1056	-8.5 (7.86)*	464	-11.3 (10.22)*	6703	-4.2 (6.89)*
71-100	5010	-1.0 (5.21)*	5875	-3.0 (6.39)*	2208	-5.5 (9.12)*	926	-6.3 (11.80)*	14019	-2.9 (7.23)*
101-200	8312	-0.4 (5.35)*	13840	-0.4 (7.07)*	4969	0.5 (10.56)*	2015	3.7 (15.32)*	29136	0.0 (8.26)
>200	551	1.6 (7.53)*	932	1.5 (9.36)*	438	6.3 (12.38)*	187	14.7 (31.82)*	2108	3.7 (13.79)*
Overall	24454	-1.1 (4.88)*	30088	-2.4 (6.89)*	10093	-3.3 (10.90)*	4116	-2.4 (17.02)*	68751	-2.1 (8.04)*

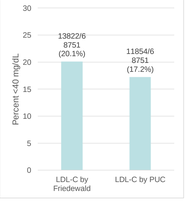
Note: Difference = calculated LDL-C – PUC LDL-C a P<0.0001 b P<0.001 P-values are from a one sample t-test performed on percent difference.

Table 5 **Percent Change from Baseline in LDL-C by Achieved Calculated LDL-C**

Lipid Parameter	LDL-C Category at Last Visit	N	% Change Mean (SD)	P-value
PUC LDL-C	<25	388	-74.4 (14.9)	<0.0001
	≥25 and <50	1208	-57.0 (18.6)	<0.0001
	≥50and <70	1433	-36.4 (25.1)	<0.0001
	≥70	7161	-6.8 (26.9)	<0.0001
	≤50	1675	-60.4 (20.1)	<0.0001
	≤70	3117	-49.0 (25.6)	<0.0001
	≤100	5712	-35.2 (29.7)	<0.0001
Calc LDL-C	<25	388	-81.2 (14.8)	<0.0001
	≥25 and <50	1208	-60.1 (19.9)	<0.0001
	≥50 and <70	1433	-37.9 (32.3)	<0.0001
	≥70	7161	-7.0 (28.9)	<0.0001
	≤50	1675	-64.3 (21.5)	<0.0001
	≤70	3117	-51.7 (30.3)	<0.0001
	≤100	5712	-36.9 (33.5)	<0.0001

* P-values are from a one sample t-test performed on percent change.

Figure 1. **Percent of values below 40 mg/dL by Friedewald and PUC**



Summary

We demonstrate:

• The accuracy of the Friedewald formula compared to PUC is very reliable when LDL-C is >100 mg/dL

• The accuracy of the Friedewald formula deteriorates as LDL-C decreases below 100 mg/dL with significant underestimation below 70 mg/dL and the percent and absolute underestimations worsen further below 50 mg/dL and 25 mg/dL

• At each threshold the discrepancy deteriorates further for each 100 mg/dL increase in triglyceride increases above 100 mg/dL

• There are several implications of using the Friedewald formula for clinical research and patient care:

- ➢ First, LDL-C reduction based on Friedewald formula to assess response to PCSK9 inhibitors, or other very effective LDL-C reducing therapy, while accurate at entry where LDL-C is usually >100 mg/dL and always >70 mg/dL, will significantly underestimate LDL-C after treatment, resulting in overestimation of the LDL-C reducing ability of the drug.
- ➢ Second, clinical and regulatory concern regarding achieving, or maintaining, patients at "too low" LDL-C, often defined as 25 or 50, based on the Friedewald formula will create a large number of "false" positives which will lead to inappropriate down-titration or discontinuation of LDL-C reducing therapies, which will be more pronounced in patients with moderately elevated TG (>100 mg/dL).

▪ In circumstances where LDL-C is less than 50 mg/dL and possibly <70 mg/dL, treatment decisions should be based on more accurate determination of LDL-C, such as PUC.

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Disclosure

None