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Comparison of Novel Equations for Estimating Low-Density Lipoprotein Cholesterol in Patients Undergoing Coronary Angiography

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Aim: The importance of precisely quantifying low-density lipoprotein cholesterol (LDL-C) has become more pronounced over the years, with the rise of metabolic syndrome in the population and the reduction in LDL-C treatment goals. This study aims to compare two novel equations indirectly estimating LDL-C and assess their compatibility with Friedewald formula, in a population with high cardiovascular risk.

Methods: This study is a retrospective analysis of the lipid profiles of 10,006 patients who underwent coronary angiography. LDL-C was calculated using Friedewald, Martin, and Sampson equations, and the compatibility between estimations was compared using methods of concordance and reclassification.

Results: Our findings show that Martin and Sampson equations displayed high rates of upward LDL-C reclassification (10.8% and 7.5%, respectively) when compared with Friedewald equation. In comparison to the Sampson method, Martin also reclassified 3.8 % of patients to a higher LDL-C category. The magnitude of discordance between LDL-C estimates was more pronounced in hypertriglyceridemic patients, and this increased progressively with the reduction in LDL-C. The proportion of patients with LDL-C <70 mg/dL reclassified to a higher LDL-C category reached 44% (Sampson vs. Friedewald), 65% (Martin vs. Friedewald), and 37% (Martin vs. Sampson) in those with triglyceride levels between 200 and 399 mg/dL.

Conclusions: Both Martin and Sampson LDL-C estimates displayed significant proportion of upward discordance with reclassification to higher LDL-C categories compared to Friedewald formula, particularly in patients with elevated triglycerides and low LDL-C, a population in whom more accurate estimation of LDL-C is required. Further studies are warranted to validate the recently developed Sampson equation with comparison to Martin method that tended to more significantly overestimate LDL-C.

Key words: Cholesterol, LDL, Triglycerides, Friedewald equation, Lipids

Introduction

Extensive evidence from epidemiologic, genetic, and clinical intervention studies have established cholesterol-rich low-density lipoprotein (LDL) as a causal factor and a primary treatment target in atherosclerotic cardiovascular disease[1)]. Nevertheless, despite the importance of precisely quantifying LDL cholesterol (LDL-C), it is not measured directly in most clinical laboratories around the world. Friedewald equation has been often used to estimate LDL-C concentration in clinical practice since the 1970s[2)]. LDL-C is calculated indirectly by Friedewald equation as total cholesterol (TC) minus high-density lipoprotein cholesterol (HDL-C) minus triglycerides (TG)/5 in milligrams per deciliter. However, the equation has been observed to underestimate LDL-C when compared to ultracentrifugation or methods of direct LDL-C measure-

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ment[3-5]. This is particularly evident in the presence of elevated TGs and/or low LDL-C levels[6]. These two main drivers, which make LDL-C estimation inaccurate, became more pronounced in recent years due to the increase in the rate of diabetes and metabolic syndrome on the one hand and the reduction of LDL-C treatment goals in high cardiovascular risk patients on the other[7]. The clinical implication of inaccurate LDL-C measurement is significant when considering whether to intensify lipid-lowering therapies beyond statins to attain lipid treatment goals. This is further underscored in the current era in which combination of statin plus ezetimibe showed greater coronary plaque regression[8] with significantly lower incidence of cardiovascular outcomes[9] and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibition reduces LDL-C to unprecedentedly low levels[10]. In addition, recent guidelines have endorsed the routine use of non-fasting lipid panels, increasing the likelihood of inaccurate LDL-C calculation due to elevated TG levels measured in the non-fasting state[11]. Since direct LDL-C measurement by preparative ultracentrifugation is time-consuming and requires specialization, and homogenous assays are costly with issues regarding reliability and standardization, the indirect estimation of LDL-C is anticipated to continue to be the customary method for LDL-C quantification[5, 12]. Therefore, there is a need for more methods to accurately estimate LDL-C levels, to aid better performance in current clinical practice.

In 2013, Martin and colleagues have developed a more complex LDL-C equation (referred to here as Martin equation) using an individualized factor (ranging from 3.1 to 9.5) to account for the heterogeneity in very-low density lipoprotein cholesterol (VLDL-C), using a 180-cell method to estimate VLDL-C according to patient-specific TG and non-HDL-C levels[13]. Martin equation was demonstrated to be more accurate in estimating LDL-C levels and better validated than other proposed alternative methods for LDL-C estimation[14-24]. However, its implementation remains limited in clinical practice, as it is less intuitive to calculate and needs to be incorporated into laboratory information systems for automated reporting. Recently, Sampson and colleagues have developed an additional novel method to estimate LDL-C values using a very large dataset, reporting good precision compared to reference methods of ultracentrifugation and direct LDL-C tests[25]. The new LDL-C equation (referred to here as Sampson equation) is based on the same laboratory methods compared with the standard lipid panel; this do not require any additional costs and was suggested to be more accurate than the previous equations, particularly in cases of hypertriglyceridemia up to 800 mg/dL and/or low LDL-C levels.

Aim

This study aims to provide a comparative analysis of the two novel equations for LDL-C estimation, and their compatibility with Friedewald formula, in a large cohort of real-world patients undergoing coronary angiography. We also seek to evaluate discordance rates between LDL-C equations, particularly in cases of mild hypertriglyceridemia and low LDL-C levels that are often observed in patients treated for atherosclerotic cardiovascular disease and better understand the future clinical utility of the novel LDL-C estimates.

Methods

Study Population

We conducted a retrospective analysis of patients insured by Clalit Health Services (CHS) who underwent coronary angiography for the evaluation and/or treatment of coronary artery disease at Carmel Medical Centre, Haifa, Israel, between 2000 and 2019. The analysis was restricted to patients of whom a full lipid profile was available in the catheterization laboratory electronic files, which document the most recent lipid profile performed prior to angiography. Excluded were all cases with TG levels >400 mg/dL to enable LDL-C estimation using Friedewald equation. A total of 10,006 patients were enrolled in this study. Demographic data, clinical variables, and risk factors were the most often prospectively collected from the patients' medical files at the time of coronary angiography. Data that was not originally collected were retrieved from computerized database of CHS. The study database has received approval from Carmel Medical Center Ethics Committee, while informed consents were no longer required because of the retrospective nature of this study.

Lipid Measurements

Lipid profiles were measured in a centralized district laboratory, with blood tests customary taken after a 12-hour prolonged fasting. Serum TC and TG levels were measured enzymatically using colorimetric method, and HDL-C was determined by a homogenous enzymatic assay, using an automated analyzer. Friedewald formula was utilized to calculate LDL-C values in mg/dL (LDL-C = TC − HDL-C − TG/5)[2]. Two additional LDL-C estimates were evaluated in the current analysis:

(a) Martin equation: LDL-C calculated as (non-HDL-C)–(TG/AF), where AF is an adjustable factor

estimated using 1 of the 180 different factors for the TG/VLDL-C ratio, according to non-HDL-C and TG levels[13]

(b) Sampson equation: LDL-C = TC/0.948 − HDL-C/0.971 − (TG/8.56 + TG × non-HDL-C/2140 − TG^2/16100) − 9.44 [25].

Data Analysis

Continuous data are reported as means and standard deviation or median and interquartile range (IQR), while categorical variables are presented as numbers and percentages. Several approaches were used to assess the compatibility between Friedewald, Martin, and Sampson LDL-C estimations. Histograms were used to visually compare the distribution of estimated LDL-C by each method. Scatterplots with calculation of Pearson's correlation coefficient were performed to assess the correlation between equations. Discordance between LDL-C estimates stratified by triglyceride categories was visually assessed using Bland and Altman plots, with calculation of absolute and percent differences[26]. Estimated LDL-C values were classified according to clinically relevant cutoffs of <70, 70–99, 100–189, and ≥ 190 mg/dL; this is to compare the level of agreement between the three methods in categorizing participants to various risk groups. Concordance in classification between LDL-C estimates was examined through cross-tabulations by LDL-C categories, in order to study the impact of reclassification. Discordance was then assessed according to proportion of subjects reclassified to a higher (upward) or lower (downward) LDL-C category, when comparing LDL-C equations. The direction of reclassification (upward vs. downward) refers to three different comparisons between methods: (i) Sampson vs. Friedewald, (ii) Martin vs. Friedewald, and (iii) Martin vs. Sampson. Additional exploratory analyses were performed according to triglyceride strata of <150, 150–199, and 200–399 mg/dL and in patients with low and very-low LDL-C levels.

SPSS statistical software version 25.0 and MedCalc version 16.8.4 were used to perform statistical analyses. Bar charts were created in Microsoft Excel 2019.

Results

Mean age was 65 ± 11 years and 71% of the patients were men. Diabetes mellitus was present in 38% and obesity in 32%, suggesting a high proportion of metabolic syndrome in the study population. Prior diagnosis of atherosclerotic cardiovascular disease was evident in 63%, and 36% presented with coronary angiography in the setting of acute coronary syndrome. Patients' baseline characteristics are presented in **Table 1**.

Histograms depicting LDL-C distribution as estimated by the three equations are presented in **Figs. 1a-1c**. Median (IQR) levels of estimated LDL-C were found to be lowest using the Friedewald equation [98 (77–123) mg/dL)], compared with results determined through Sampson [102 (81–127) mg/dL] and Martin [103 (83–127) mg/dL] equations. A strong positive correlation was detected between LDL-C estimates using the different methods as shown in the scatterplots in **Figs. 1d-1f**. LDL-C levels <70 mg/dL were noted in 16.4%, 13.4%, 12.2% and LDL-C ≥ 190 mg/dL in 1.63%, 1.82%, 1.84% of patients using Friedewald, Sampson, and Martin equations, respectively.

Overall concordance between LDL-C categories (<70, 70–99, 100–189, ≥ 190 mg/dL) was found to be lowest when comparing Martin and Friedewald equations (88.4%), higher between Sampson and Friedewald (92.3%), and highest comparing Martin and Sampson (95.1%) equations **(Table 2)**. Compared with Friedewald equation, both Martin and Sampson equations showed high proportion of upward reclassification (10.8% and 7.5%, respectively) and low proportion of downward reclassification (0.7% and 0.2%, respectively). Compared to Sampson method, Martin equation reclassified 3.8% of patients to a higher LDL-C category and 1.1% to a lower category.

The magnitude of the difference between LDL-C estimates was progressively higher with the increase in TG concentration **(Supplemental Fig. 1)**. In patients with TGs between 200 and 399 mg/dL, the mean relative change in LDL-C was determined to be at +8.1%, +13.7%, and +5.7% for Sampson vs. Friedewald, Martin vs. Friedewald, and Martin vs. Sampson equations, compared to +2.2%, +1.5%, and −0.7%, respectively, in those with normal TG levels <150 mg/dL **(Table 3)**. The gap between LDL-C estimates in hypertriglyceridemic patients increased gradually with the reduction in LDL-C levels, as demonstrated by Bland-Altman plots in **Fig. 2**.

The proportion of patients with low LDL-C < 70 mg/dL reclassified to a higher LDL-C category increased across TG strata, reaching 44% (Sampson vs. Friedewald), 65% (Martin vs. Friedewald), and 37% (Martin vs. Sampson) in those with TG levels between 200 and 399 mg/dL **(Fig. 3)**. Scatterplots displaying the correlation between LDL-C estimates, specifically in patients with both LDL-C <70 mg/dL and triglycerides ≥ 150 mg/dL, are shown in **Supplemental Fig. 2**. The overall concordance between

Table 1. Baseline patients characteristics

Variable	Study population: $n = 10,006$
Age (years)	65 ± 11
Men	7063 (70.6%)
Obesity	3160 (31.6%)
Hypertension	7282 (72.8%)
Hyperlipidemia	7266 (72.6%)
Active smoker	1875 (18.7%)
Diabetes mellitus	3785 (37.8%)
Chronic Kidney disease	866 (8.7%)
Acute coronary syndrome	3604 (36.0%)
Prior myocardial infarction	4854 (48.5%)
Prior CABG	1002 (10.0%)
Prior stroke	557 (5.6%)
Peripheral artery disease	1079 (10.8%)
Prior cancer	1053 (10.5%)
Heart failure	1530 (15.3%)
Total cholesterol (mg/dL)	mean ± SD 177 ± 41; median (IQR) 173 (148-202)
HDL-C (mg/dL)	mean ± SD 44 ± 12; median (IQR) 42 (36-50)
Triglycerides (mg/dL)	mean ± SD 149 ± 64; median (IQR) 137 (101-186)
Non-HDL-C (mg/dL)	mean ± SD 133 ± 39; median (IQR) 128 (104-157)
LDL-C (mg/dL), Friedewald equation	mean ± SD 102 ± 35; median (IQR) 98 (77-123)

CABG, coronary artery bypass graft surgery; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol

LDL-C categories according to TG strata (<150; 150–199; 200–399 mg/dL) is presented in **Supplemental Table 1**.

Patients with diabetes were determined to have higher rates of hypertriglyceridemia (TG >150 mg/dL) compared to those without diabetes (49.1% versus 37.3%, respectively). Accordingly, the proportion of patients with low LDL-C <70 mg/dL reclassified to any higher LDL-C category was somewhat higher when compared with patients without diabetes (Martin vs. Friedewald, diabetes 29.2% vs. non-diabetes 26.2%; Sampson vs. Friedewald, diabetes 19.8% vs. non-diabetes 17.9%; and Martin vs. Sampson, diabetes 11.7% vs. non-diabetes 9.9%).

As very-low LDL-C levels are more commonly observed in clinical care due to the reduction in treatment targets by recent dyslipidemia guidelines and the use of PCSK9 inhibition, we further analyzed the concordance among the three equations at very-low LDL-C categories **(Supplemental Table 2)**. In patients with Friedewald estimation of LDL-C <40 mg/dL, 42% and 54% were reclassified to a higher LDL-C category using the Sampson and Martin equations, respectively; moreover, 21% of patients with estimated LDL-C <40 mg/dL as per Sampson equation were reclassified to a higher LDL-C category using Martin equation.

Discussion

In this current study, we have compared the performance of Friedewald formula with two novel methods for LDL-C estimation using a sample of 10,006 patients, who underwent coronary angiography for evaluation and treatment of coronary artery disease. Only patients with mild hypertriglyceridemia (<400 mg/dL) were included. Both Martin and, the recently developed, Sampson equations showed high proportion of upward discordance compared with Friedewald formula. The difference between LDL-C estimates was more pronounced in hypertriglyceridemic patients, and this increased progressively with the reduction in LDL-C levels, resulting in high proportion of these patients to be reclassified to a higher LDL-C category, using the other two novel equations. Moreover, compared to the recently developed Sampson equation, Martin tended to overestimate LDL-C levels, particularly in patients with hypertriglyceridemia and low LDL-C values.

Preparative ultracentrifugation is considered the standard reference method for LDL-C measurement[5]. However, as it has been considered as time-consuming and requires a large serum volume, this method has not been routinely performed in clinical laboratories. Accordingly, the indirect LDL-C estimation of Friede-

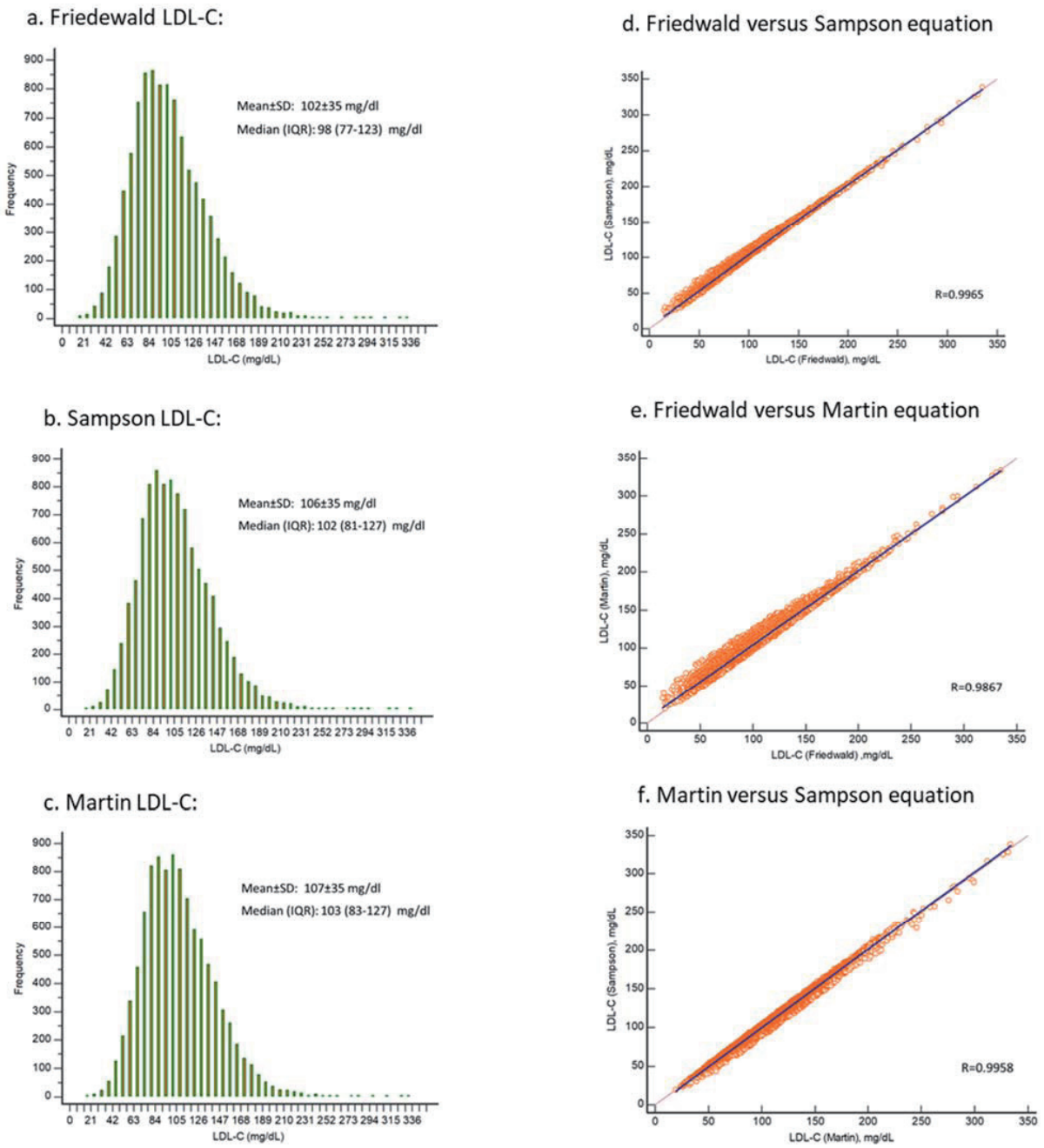

Fig. 1. Histogram and scatterplot of estimated LDL-C levels, comparing the three equations

Line of equality is shown as dashed line and line of regression shown as solid line; R = orrelation coefficient.

wald *et al.* became the method of choice for routine LDL-C calculation[2]. Nevertheless, Friedewald formula is not applicable to plasma containing TGs > 400 mg/dL; this requires fasting and was demonstrated by several studies to underestimate LDL-C when TG concentrations are high and/or LDL-C levels are low[3-5, 27, 28]. Direct LDL-C measurement by homogenous assays was developed over the years to cope with these limitations, showing improved precision and accuracy compared to indirect LDL-C estimations[29-31]. However, they are costly with poor standardization, and their reliability and specificity for LDL in the presence of abnormal lipoproteins are still questionable[5, 12]. Therefore, in many clinical laboratories worldwide, the sole method used for LDL-C quantification is still the Friedewald equation; this is also the case in CHS, a non-profit healthcare provider covering about a half of the Israeli population, includ-

Table 2. Concordance between LDL-C categories according to the different LDL-C equations

a. Sampson versus Friedewald equation

		Sampson LDL-C (mg/dL)				Total
		<70	70-99	100-189	≥ 190	
Friedewald LDL-C (mg/dL)	<70	1326	312	0	0	1638
	70-99	14	3087	417	0	3518
	100-189	0	2	4664	21	4687
	≥ 190	0	0	0	163	163
Total		1340	3401	5081	184	10006

Sampson compared to Friedewald LDL-C: overall concordance 9240/10006 = 92.3%;
Discordance: upward 750/10006 = 7.5%, downward 16/10006 = 0.2%

b. Martin versus Friedewald equation

		Martin LDL-C (mg/dL)				Total
		<70	70-99	100-189	≥ 190	
Friedewald LDL-C (mg/dL)	<70	1179	459	0	0	1638
	70-99	38	2876	604	0	3518
	100-189	0	34	4633	20	4687
	≥ 190	0	0	1	162	163
Total		1217	3369	5238	182	10006

Martin compared to Friedewald LDL-C: overall concordance 8850/10006 = 88.4%;
Discordance: upward 1083/10006 = 10.8%, downward 73/10006 = 0.7%

c. Martin versus Sampson equation

		Martin LDL-C (mg/dL)				Total
		<70	70-99	100-189	≥ 190	
Sampson LDL-C (mg/dL)	<70	1193	147	0	0	1340
	70-99	24	3153	224	0	3401
	100-189	0	69	5001	11	5081
	≥ 190	0	0	13	171	184
Total		1217	3369	5238	182	10006

Martin compared to Sampson LDL-C: overall concordance 9518/10006 = 95.1%; discordance: upward 382/10006 = 3.8%; downward 106/10006 = 1.1%
Shown are absolute numbers in each category. White cells represent concordance or total values; colored cells represent upward or downward discordance.

Table 3. Absolute differences between LDL-C estimates, according to triglyceride groups

LDL-C equations	Differences* between LDL-C estimates	Triglycerides <150 mg/dL	Triglycerides 150-199 mg/dL	Triglycerides 200-399 mg/dL
LDL-C (Sampson-Friedwald)	Difference (mg/dl)	2.1 (-1.0–5.3)	4.8 (1.5–8.1)	7.3 (0.8–13.8)
	Delta (%)	2.2% (-1.7%–6.1%)	5.3% (-1.3%–11.8%)	8.1% (-3.9%–20.1%)
LDL-C (Martin-Friedwald)	Difference (mg/dl)	1.2 (-3.6–5.9)	6.5 (1.0–11.9)	13.1 (2.6–23.5)
	Delta (%)	1.5% (-4.7%–7.7%)	7.3% (-3.1%–17.7%)	13.7% (-4.7%–32.1%)
LDL-C (Martin-Sampson)	Difference (mg/dl)	-1 (-4.1–2.1)	1.6 (-1.4–4.7)	5.8 (0.3–11.2)
	Delta (%)	-0.7% (-3.8%–2.4%)	2% (-2.4%–6.4%)	5.7% (-1.7%–13%)

*numbers present in mg/dL or percentage the average differences between LDL-C equations ± 1.96 standard deviations of the difference

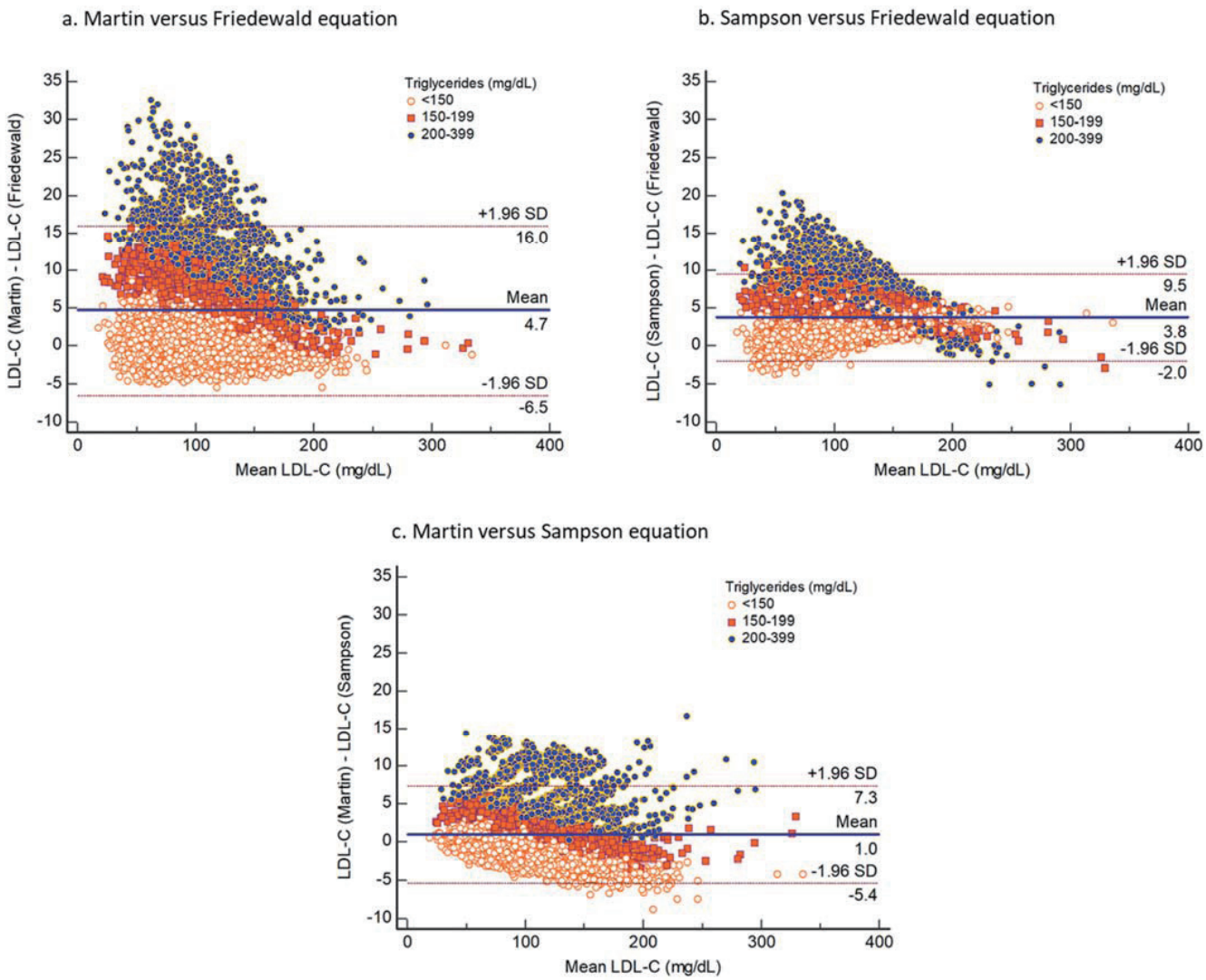

Fig. 2. Bland-Altman plots for the comparison of estimated LDL-C equations, stratified by triglyceride levels

Horizontal lines present the average differences (mg/dL) between LDL-C equations ± 1.96 standard deviations of the difference, in the overall population. Stratification according to triglyceride subgroups (< 150, 150–199, 200–399 mg/dL) is presented in Table 3

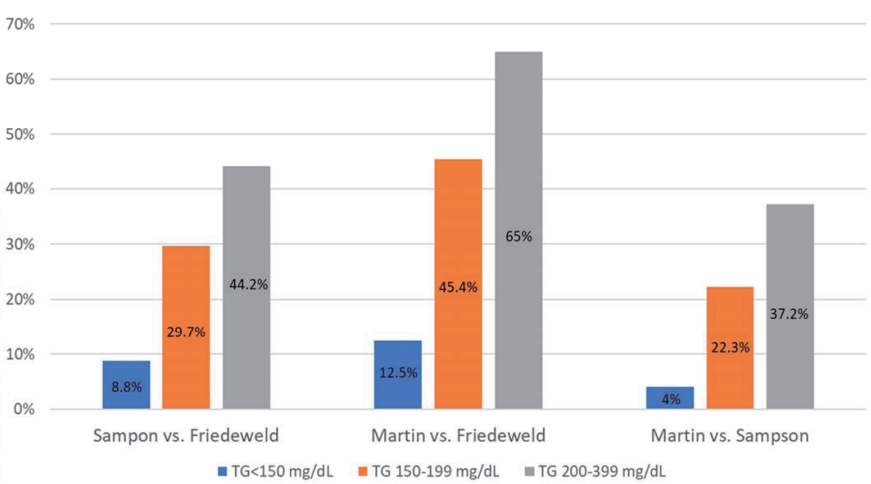

Fig. 3. Proportion of patients with low LDL-C < 70 mg/dL reclassified to a higher LDL-C category, according to LDL-C equations and triglyceride strata

TG, triglycerides

ing the current study cohort.

In 2013, Martin and colleagues developed a novel LDL-C equation using an individualized factor to account for heterogeneity in the TG to VLDL-C ratio, demonstrating improved accuracy of LDL-C estimation at high TG and low LDL-C concentrations compared to the Friedewald formula[13]. Their results were confirmed in various samples of different nationalities and clinical scenarios[14-24]. Our data is also consistent with these observations in a real-world high-risk population of patients undergoing coronary angiography, with significant upward discordance when compared with Friedewald equation, particularly noted in those with hypertriglyceridemia and low LDL-C. Recently, Sampson and colleagues have also developed a new method to indirectly estimate LDL-C levels, reporting good precision compared to ultracentrifugation and increasing the accuracy of LDL-C measurement in persons with TG values up to 800 mg/dL[25]. The authors further reported that their novel equation is at least equivalent or even more accurate than the other equations used for patients with normolipidemia and low LDL-C levels. The current results do show that similar to Martin estimation, Sampson equation significantly overestimates LDL-C levels when compared with Friedewald equation. However, comparing both novel equations, Martin was observed to overestimate the LDL-C levels calculated using the Sampson equation, particularly in patients with hypertriglyceridemia and low LDL-C values. To the best of our knowledge, our study is the first to compare both novel LDL-C estimates in a real-world cohort of high cardiovascular risk patients undergoing coronary angiography.

As LDL-C is a major causative risk factor and a primary treatment target in atherosclerotic cardiovascular disease, it is important to precisely estimate LDL-C[1, 7, 32]. Misclassification of risk may have negative consequences when allocating preventive therapies that are proven in reducing cardiovascular outcomes. In this cohort of patients of whom most are considered to be at very-high cardiovascular risk, about half of those with LDL-C <70 mg/dL and mildly elevated TGs were reclassified to a higher LDL-C category using both Martin and Sampson equations. Similar findings were observed in patients with very-low LDL-C <40 mg/dL. The results provide additional evidence that the standard use of Friedewald equation in clinical practice may lead to misclassification of high-risk individuals and to potential underutilization of lipid-lowering therapies, particularly when low levels of LDL-C and mild hypertriglyceridemia concomitantly exist. This scenario is anticipated to be encountered more often in clinical care with the implementation of recent recommendations of the European Society of Cardiology, advising to reduce LDL-C treatment goals to <55 mg/dL and even <40 mg/dL in patients categorized at very-high cardiovascular risk[7]. Nonetheless, it should be noted that from a historical perspective, landmark trials such as the Scandinavian Simvastatin Survival Study (4S), which showed reduced morbidity and mortality with statin therapy, have estimated LDL-C using Friedewald equation and not by direct LDL-C measurement[33]. This was also the case in the Collaborative Atorvastatin Diabetes Study in which ultracentrifugation was performed in type 2 diabetic patients only when TGs were between 355 and 600 mg/dL, exposing patients with mild hypertriglyceridemia to inaccurate LDL-C estimation[34]. Accordingly, whether the use of novel equations for reporting indirect LDL-C will improve risk estimation and cardiovascular outcomes remains a question that is needed to be answered[35].

In recent years, the use of PCSK9 monoclonal antibodies enabled many high-risk patients to achieve profound reduction in LDL-C. Large outcome trials with PCSK9 inhibitors did not find any lower limit to the association between lessening of LDL-C and the reduction in cardiovascular events in patients with proven cardiovascular disease[36, 37]. As Friedewald equation is prone to underestimate LDL-C particularly in patients with very-low LDL-C, the FOURIER trial evaluating evolocumab in patients with known cardiovascular disease used preparative ultracentrifugation method for LDL-C estimation when Friedewald LDL-C was observed to be at <40 mg/dL[36]. Recently, Martin and colleagues have investigated the accuracy of LDL-C measurements in the FOURIER trial, specifically in PCSK9 inhibitor-treated patients achieving very-low LDL-C levels <40 mg/dL; their results showed that Martin method more closely resembled ultracentrifugation than the Friedewald approach, suggesting it should be the preferred method to estimate LDL-C levels in such intensively treated patients[38]. Our findings are in line with these results showing significant upward discordance using the two novel LDL-C equations compared to Friedewald estimates in similar patients at high cardiovascular risk and very-low LDL-C under 40 mg/dL. Furthermore, to a lower extent, Martin method overestimated LDL-C also in comparison to the Sampson equation in this group of patients with very-low LDL-C levels, highlighting the need for further studies to evaluate the compatibility of both methods with ultracentrifugation and direct LDL-C measurement.

Several limitations of this study should be noted. First, direct LDL-C measurements are not routinely performed in clinical laboratories in Israel. Therefore,

due to the retrospective nature of this study, we could not determine the inaccuracy in estimating LDL-C in comparison to direct measurement of LDL-C. Nevertheless, the current findings regarding the underestimation of LDL-C using Friedewald formula are compatible with the previous observations in the literature, and our study further adds, for the first time, important real-world data on the performance of the recently developed Sampson equation compared to both the Friedewald and Martin estimations. Second, we examined a single measurement of LDL-C and not serial measurements which may have resulted in misclassification of the study participants. We also did not have data on lipid-modifying agents, which may have affected the results. Third, although patients are routinely directed to perform lipid panel in the fasting state, we cannot ensure that all blood tests were performed during prolonged fasting. Fourth, the current analysis was limited to patients with TGs of less than 400 mg/dL, and therefore we did not estimate LDL-C in patients with moderate hypertriglyceridemia in which Sampson equation was reported to be more accurate than other indirect methods. Finally, we did not assess how elevated lipoprotein(a) levels or type III hyperlipoproteinemia may have influenced LDL-C estimation using the different equations.

Conclusions

To conclude, in a cohort of high-risk patients undergoing coronary angiography, both Martin and Sampson equations for LDL-C estimation displayed significant proportion of upward discordance with reclassification to higher LDL-C categories compared to the Friedewald formula, particularly observed in patients with elevated TGs and low LDL-C. In this population, the use of novel equations for indirect LDL-C estimation, with automated reporting by laboratory systems, should be even more considered. However, additional studies are warranted in order to validate the accuracy and clinical utility of the new Sampson LDL-C equation in various clinical scenarios with comparison to the performance of both Martin estimation and direct methods for LDL-C quantification.

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None.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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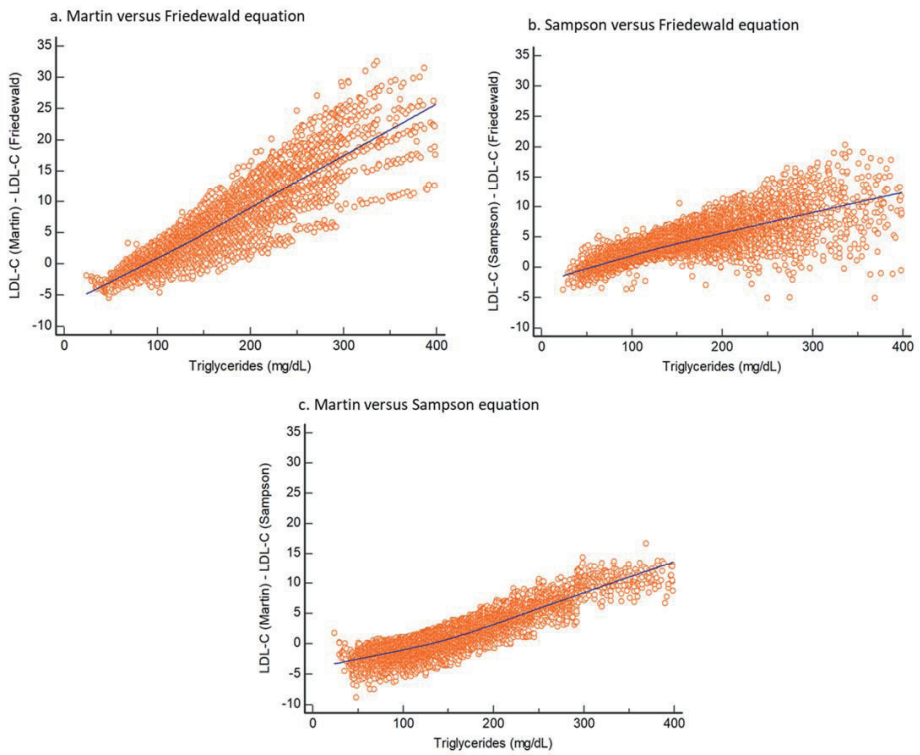
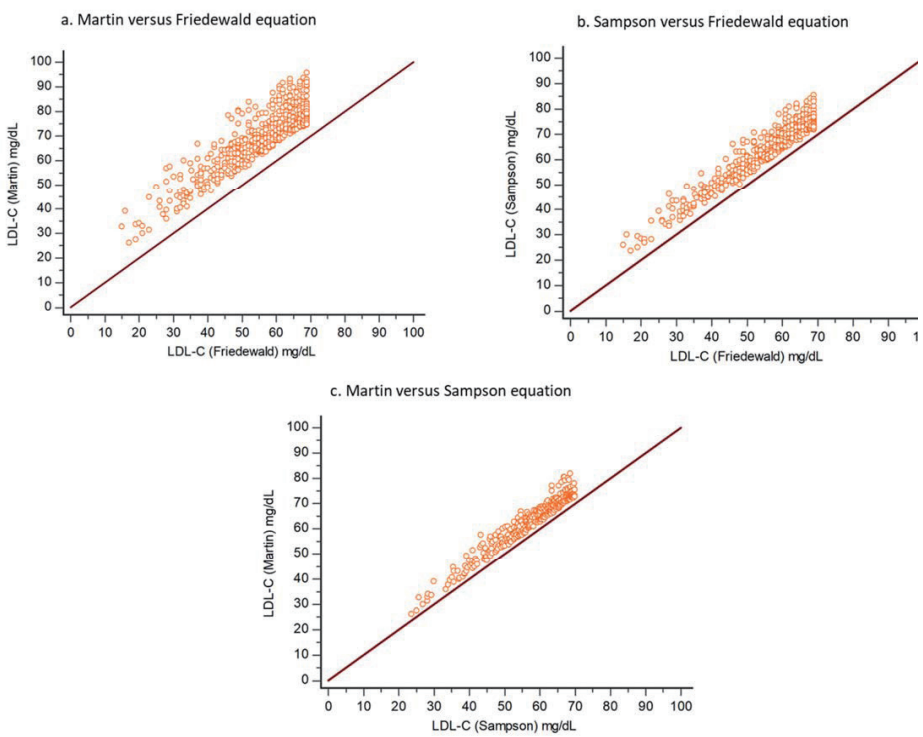


Supplemental Fig. 1. LDL-C differences between equations across triglyceride concentrations
Solid line presents trend with LOESS smoothing span of 80%



Supplemental Fig. 2. Scatterplots displaying correlation between LDL-C estimates in patients with both LDL-C <70mg/dL and triglycerides ≥ 150 mg/dL (horizontal axis)
Horizontal axis presents only patients with both LDL-C <70 mg/dL and triglycerides >150 mg/dL. Solid line presents equality line.

Supplemental Table 1. Concordance between LDL-C categories, according to LDL-C equations and triglyceride strata

a. Sampson versus Friedewald LDL-C

Triglycerides < 150 mg/dL (*n* = 5,829, 58%)

		Sampson LDL-C (mg/dL)				Total
		< 70	70-99	100-189	≥ 190	
Friedewald LDL-C (mg/dL)	< 70	947	91	0	0	1038
	70-99	14	2067	138	0	2219
	100-189	0	2	2492	7	2501
	≥ 190	0	0	0	71	71
Total		961	2160	2630	78	5829

Triglycerides 150-199 mg/dL (*n* = 2,170, 22%)

		Sampson LDL-C (mg/dL)				Total
		< 70	70-99	100-189	≥ 190	
Friedewald LDL-C (mg/dL)	< 70	215	91	0	0	306
	70-99	0	590	110	0	700
	100-189	0	0	1116	8	1124
	≥ 190	0	0	0	40	40
Total		215	681	1226	48	2170

Triglycerides 200-399 mg/dL (*n* = 2,007, 20%)

		Sampson LDL-C (mg/dL)				Total
		< 70	70-99	100-189	≥ 190	
Friedewald LDL-C (mg/dL)	< 70	164	130	0	0	294
	70-99	0	430	169	0	599
	100-189	0	0	1056	6	1062
	≥ 190	0	0	0	52	52
Total		164	560	1225	58	2007

b. Martin versus Friedewald LDL-C

Triglycerides < 150 mg/dL (*n* = 5,829, 58%)

		Martin LDL-C (mg/dL)				Total
		< 70	70-99	100-189	≥ 190	
Friedewald LDL-C (mg/dL)	< 70	909	129	0	0	1038
	70-99	38	2060	121	0	2219
	100-189	0	34	2466	1	2501
	≥ 190	0	0	1	70	71
Total		947	2223	2588	71	5829

Triglycerides 150-199 mg/dL (*n* = 2,170, 22%)

		Martin LDL-C (mg/dL)				Total
		< 70	70-99	100-189	≥ 190	
Friedewald LDL-C (mg/dL)	< 70	167	139	0	0	306
	70-99	0	508	192	0	700
	100-189	0	0	1122	2	1124
	≥ 190	0	0	0	40	40
Total		167	647	1314	42	2170

(Cont. Supplemental Table 1)

Triglycerides 200-399 mg/dL (n = 2,007, 20%)

		Martin LDL-C (mg/dL)				Total
		<70	70-99	100-189	≥ 190	
Friedewald LDL-C (mg/dL)	<70	103	191	0	0	294
	70-99	0	308	291	0	599
	100-189	0	0	1045	17	1062
	≥ 190	0	0	0	52	52
Total		103	499	1336	69	2007

c. Martin versus Sampson LDL-C

Triglycerides <150 mg/dL (n = 5,829 , 58%)

		Martin LDL-C (mg/dL)				Total
		<70	70-99	100-189	≥ 190	
Sampson LDL-C (mg/dL)	<70	923	38	0	0	961
	70-99	24	2116	20	0	2160
	100-189	0	69	2561	0	2630
	≥ 190	0	0	7	71	78
Total		947	2223	2588	71	5829

Triglycerides 150-199 mg/dL (n = 2,170, 22%)

		Martin LDL-C (mg/dL)				Total
		<70	70-99	100-189	≥ 190	
Sampson LDL-C (mg/dL)	<70	167	48	0	0	215
	70-99	0	599	82	0	681
	100-189	0	0	1226	0	1226
	≥ 190	0	0	6	42	48
Total		167	647	1314	42	2170

Triglycerides 200-399 mg/dL (n = 2,007, 20%)

		Martin LDL-C (mg/dL)				Total
		<70	70-99	100-189	≥ 190	
Sampson LDL-C (mg/dL)	<70	103	61	0	0	164
	70-99	0	438	122	0	560
	100-189	0	0	1214	11	1225
	≥ 190	0	0	0	58	58
Total		103	499	1336	69	2007

Shown are absolute numbers in each category. White cells represent concordance or total values; colored cells represent upward or downward discordance, respectively

Supplemental Table 2. Concordance between Very-Low LDL-C groups according to the different LDL-C equations

a. Sampson versus Friedewald equation

		Sampson LDL-C (mg/dL)					Total
		<25	25 to <40	40 to <50	50 to <70	≥ 70	
Friedewald LDL-C (mg/dL)	<25	4	9	0	0	0	13
	25 to <40	0	57	48	2	0	107
	40 to <50	0	8	146	101	0	255
	50 to <70	0	0	7	944	312	1263
	≥ 70	0	0	0	14	8354	8368
Total		4	74	201	1061	8666	10006

b. Martin versus Friedewald equation

		Martin LDL-C (mg/dL)					Total
		<25	25 to <40	40 to <50	50 to <70	≥ 70	
Friedewald LDL-C (mg/dL)	<25	2	10	1	0	0	13
	25 to <40	0	43	40	24	0	107
	40 to <50	0	7	117	125	6	255
	50 to <70	0	0	8	802	453	1263
	≥ 70	0	0	0	38	8330	8368
Total		2	60	166	989	8789	10006

c. Martin versus Sampson equation

		Martin LDL-C (mg/dL)					Total
		<25	25 to <40	40 to <50	50 to <70	≥ 70	
Sampson LDL-C (mg/dL)	<25	2	2	0	0	0	4
	25 to <40	0	58	16	0	0	74
	40 to <50	0	0	147	54	0	201
	50 to <70	0	0	3	911	147	1061
	≥ 70	0	0	0	24	8642	8666
Total		2	60	166	989	8789	10006

Shown are absolute numbers in each category. White cells represent concordance or total values; colored cells represent upward or downward discordance, respectively