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Comparison of Equations for the Calculation of LDL-Cholesterol in Familial Hypercholesterolemia: Data from Iranian Registry

Ailesel Hiperkolesterolemide LDL-Kolesterol Hesaplaması için Denklemlerin Karşılaştırılması: İran Kayıtlarından Elde Edilen Veriler

ORIGINAL ARTICLE
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ABSTRACT

Objective: Low-density lipoprotein cholesterol is the mainstay of diagnosis, treatment, and follow-up of patients with familial hypercholesterolemia, the most prevalent autosomal dominant disorder among humans. Since the reference measurement method (ultracentrifugation) is time-consuming and expensive, many formulas emerged to calculate low-density lipoprotein cholesterol levels and are commonly used in laboratories.

Methods: To compare the performance of 3 low-density lipoprotein cholesterol calculation equations with a direct method (enzymatic photometric assay), the lipid profiles of 1148 patients of the registry of familial hypercholesterolemia in Iran were analyzed retrospectively, 270 of which had a possible or definite familial hypercholesterolemia diagnosis according to Dutch criteria. While measured using the direct method, we calculated the low-density lipoprotein cholesterol levels using the Friedewald, Chen, and Anandaraja formulas.

Results: Our results showed that all 3 formulas are highly correlated with the direct method, and the Chen formula showed the highest intra-class correlation coefficient among all (0.954 among all patients with hypercholesterolemia and 0.947 among the familial hypercholesterolemia population). In addition, the Chen formula was the most sensitive, and the Friedewald formula was the most specific formula using a low-density lipoprotein cholesterol cut-off of 100 in familial hypercholesterolemia patients.

Conclusion: Our findings encourage applying the Chen formula in addition to the Friedewald formula to make better clinical decisions for familial hypercholesterolemia patients.

Keywords: LDL-cholesterol, familial hypercholesterolemia, equations, calculation

ÖZET

Amaç: Düşük yoğunluklu lipoprotein kolesterol (LDL-C), insanlar arasında en yaygın otozomal dominant bozukluk olan ailesel hiperkolesterolemili (AH) hastaların tanı, tedavi ve takibinin temelidir. Referans ölçüm yöntemi (ultrasantrifüjleme) zaman alıcı ve pahalı olduğundan, LDL-C seviyelerini hesaplamak için birçok formül ortaya çıkmış ve laboratuvarlarda yaygın olarak kullanılmaktadır.

Yöntemler: Üç LDL-C hesaplama denkleminin performansını direkt bir yöntemle (enzimatik fotometrik test) karşılaştırmak için, İran'da AH kaydına sahip 1148 hastanın Lipid profilleri geriye dönük olarak analiz edildi, bunların 270'i Hollanda kriterlerine göre muhtemel veya kesin AH tanısı almıştı. Direkt yöntem kullanılarak ölçümün yanı sıra, LDL-C seviyelerini Friedewald, Chen ve Anandaraja formüllerini kullanarak hesapladık.

Bulgular: Sonuçlarımız, her üç formülün de direkt yöntem ile oldukça ilişkili olduğunu ve Chen formülünün, tümü arasında en yüksek sınıf içi korelasyon katsayısını gösterdiğini gösterdi (hiperkolesterolemili tüm hastalar arasında 0.954 ve AH popülasyonu arasında 0.947). Ayrıca, Chen formülü en duyarlı olandı ve Friedewald formülü, AH hastalarında 100'lük bir LDL-C cut-off kullanan en spesifik formüldü.

Sonuç: Bulgularımız, AH hastaları için daha iyi klinik kararlar almak için Friedewald formülüne ek olarak Chen formülünün uygulanmasını teşvik etmektedir.

Anahtar Kelimeler: LDL-kolesterol, ailesel hiperkolesterolemi, denklemler, hesaplama

Familial hypercholesterolemia (FH) is the most prevalent autosomal dominant disorder, affecting 1 in every 313 individuals characterized by elevated concentrations of low-density lipoprotein cholesterol (LDL-C).[1] The life-long burden of extreme LDL-C leads to accelerated atherosclerosis and cardiovascular disease (CVD).[2] The primary goal in treating FH is to decrease the cardiovascular risk, which is achieved by lowering LDL-C levels to <70 and <55 mg/dL according to new guidelines if CVD is present.[3,4]

Low-density lipoprotein cholesterol is the principal biochemical parameter for clinical diagnosis and management of FH. LDL-C levels of 190 or more in patients younger than 20, 220 or more in patients between the age of 20 and 29, and 250 or more in patients over 30 are strongly suggestive of FH.[2,5] These numbers are calculated by the commonly used Friedewald formula as total cholesterol (TC)—high-density lipoprotein cholesterol (HDL-C)—triglycerides (TG). The Friedewald formula is predominantly used in medical laboratories for estimating LDL-C levels, obviating the need for the labor-intensive and expensive method of ultracentrifugation.[6] Nevertheless, the use of a fixed factor of 5 as TG to the very-low-density lipoprotein cholesterol (VLDL-C) ratio overlooks the variance across different concentrations of TG and non-HDL-C.[7] Thus, this formula is considered inaccurate in patients with very low LDL-C levels, very high or very low TG levels, or comorbidities such as diabetes mellitus.[8,9] Many other equations have been developed to overcome the shortcomings of the Friedewald formula, including the Anandaraja and Chen formulas, but none is precise under all circumstances. The Anandaraja formula was derived from linear regression analysis in 2005 as LDL-C=(0.9 × TC) – (0.9 × [TG/5]) – 28 and was more precise in the Indian population.[10] The Chen formula was developed in 2010 as LDL-C=(0.9 × TC) – (0.9 × HDL-C) – (0.1 × TG) in a study of 2180 adults and showed closer results to the direct assay, particularly in hypertriglyceridemia.[11]

Since LDL-C plays a fundamental role in the screening, diagnosis, treatment, and follow-up of FH patients, its precise measurement is of utmost importance. In this study, we compared LDL-C calculated by the Friedewald, Anandaraja, and Chen formulas with LDL-C measured by direct homogenous assay in a large sample of FH patients. The results will propose an accurate formula in FH patients of different TG subgroups.

Methods

Study Design

This study was approved in June 2020 by the Ethics Committee of Isfahan University of Medical Sciences under approval number: 297182. The rationale and design of cascade screening and registry of FH in Iran have been previously reported.[12] Briefly, all patients over 2 years of age who had high LDL-C levels (LDL-C levels above 190 or 150 under pharmacological treatment) who were referred from laboratories or health centers or attended referral cardiovascular centers for percutaneous coronary interventions due to premature (men under 55 and women under 65) coronary artery disease were included. All participants gave informed consent and filled out a questionnaire including demographic data and drug history. Patients were diagnosed with FH using The Dutch Lipid Clinic Network Score (DLCNS) of 6 or above which means probable or definite FH. Exclusion criteria include secondary hyperlipidemia or other genetic hyperlipidemia or any. Lipid profiles whether measured directly or calculated by formulas of 1148 patients were used in our analysis.

Lipid Analysis

Blood samples were obtained after an overnight fast. Lipid concentrations (TC, HDL-C, LDL-C, and TG) were measured by the homogenous enzymatic photometric method using an automatic chemistry analyzer (Hitachi 902, Roche® Basel, Switzerland) and Parsazma kits (Tehran, Iran). TC was measured by hydrolyzing cholesterol esters, oxidizing OH groups, and finally quantifying hydrogen peroxide produced in the latter reaction. Similarly, hydrogen peroxide resulted from a glycerol oxidase reaction in triglyceride measurement. HDL-C and LDL-C were measured by enzymatic photometric assays after removing other lipoproteins by blocking reagents.[13] Indirect LDL-C is estimated using formulas shown in Table 1.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) software ver. 21.00 for Windows (IBM Corp., Armonk, NY, USA) was used for data analyses. Data were categorized into 4 groups based on TG concentrations, and the sensitivity and specificity of each formula for identifying LDL-C ≥ 100 mg/dL were calculated. Additionally, the correlation of calculated and directly measured LDL-C values was tested by Pearson's correlation in each TG subgroup. The intra-class correlation coefficient (ICC) was reported by 2-way mixed analysis to investigate the agreement between different LDL calculation methods and the direct method. Trend and Common Bland–Altman plots expressed the agreement and absolute difference between the 3 formulas and the directly measured LDL-C. A *P* value less than .05 was considered statistically significant.

Results

A total of 1148 patients (980 males) were included in this study, 279 of which had probable or definite FH diagnosis using DLCNS. The mean age of the participants at the time of enrollment was 51.3. Other demographic data of participants were reported in previous reports R. The mean age, lipid values, and calculated

ABBREVIATIONS

CVD	Cardiovascular disease
DLCNS	Dutch Lipid Clinic Network Score
FH	Familial hypercholesterolemia
HDL-C	High-density lipoprotein cholesterol
ICC	Intra-class correlation coefficient
LDL-C	Low-density lipoprotein cholesterol
TC	Total cholesterol
TG	Triglycerides
VLDL-C	Very-low-density lipoprotein cholesterol

Table 1. Formulas Used to Calculate LDL-C

Formula	Equation
Friedewald[6]	LDL-C=TC – HDL-C – (TG/5)
Chen[11]	LDL-C=(0.9 × TC) – (0.9 × HDL-C) – (0.1 × TG)
Anandaraja[10]	LDL-C=(0.9 × TC) – (0. 9 × [TG/5]) – 28

LDL-C, low-density lipoprotein cholesterol.

Table 2. Age, Lipid Profile, and Calculated LDL-C Results

	Possible, Probable, or Definite	Probable or Definite FH Patients
Age (year)	51.3 ± 12.2	50.9 ± 14.2
TG (mg/dL)	158.3 ± 68.2	163.0 ± 70.7
TC (mg/dL)	208.7 ± 70.8	246.4 ± 89.2
HDL-C (mg/dL)	47.2 ± 11.6	48.6 ± 12.0
LDL-C, Direct measurement (mg/dL)	124.0 ± 53.6	152.8 ± 69.3
LDL-C, Anandaraja (mg/dL)	131.5 ± 62.1	164.4 ± 80.0
LDL-C, Chen (mg/dL)	129.7 ± 58.6	161.7 ± 74.5
LDL-C, Friedewald (mg/dL)	130.0 ± 64.4	165.1 ± 82.8

Data are shown as mean ± SD.
TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; FH, familial hypercholesterolemia.

LDL-C are summarized in Table 2. LDL-C levels were higher when calculated by all formulas than their actual direct measurement.

Table 3 presents the lipid profiles were divided into 4 groups considering the TG levels. The sensitivity and specificity of each formula using an LDL-C cut-off of 100 mg/dL are presented in Table 3. Chen formula had the highest sensitivity and specificity for detecting LDL-C levels above 100 among all patients. However, the Friedewald formula was more specific in patients with probable or definite FH. In TG levels between 51 and 150, Anandaraja and Chen formulas were 98.5% sensitive among all patients, with the Chen formula being more specific than the other 2 formulas. Among FH patients of the same TG range, the Anandaraja formula was 100% sensitive but only 67.6% specific. In TG levels between 151 and 200 and above 200, the Chen formula was the most sensitive, and the Friedewald formula was the most specific. In FH patients of the same TG range, the Fridewald formula showed the best overall performance.

The results of regression analyses of direct and estimated LDL-C are reported in Table 4. All 3 formulas had high correlations with the direct method. The Chen formula had the highest ICC, and therefore the strongest correlation with the direct measurement. In FH patients of all TG levels, the Chen formula had the highest correlation with the direct method, except for TG levels between 151 and 200, in which the Friedewald formula exhibited a higher ICC. All 3 formulas showed the least ICCs in TG >200 mg/dL of all patients and TG between 151 and 200 of FH patients. The Bland–Altman plots of the direct LDL-C and LDL-C estimated by the 3 formulas of the overall population and FH patients are shown in Figures 1 and 2, respectively.[5]

Discussion

In the present study, the LDL-C levels of 1148 FH registered patients in Iran have been calculated using Friedewald, Chen, and Anandaraja formulas then compared to the results of direct measurement. Among patients with possible or definite FH diagnosis in our study, the Friedewald formula outperformed the Chen formula only in TG levels between 150 and 2000ur results showed that all 3 formulas highly correlate with direct measurement among all patients, with the Chen formula showing the highest ICC (0.954 compared to 0.939 of Friedewald and 0.933 of Anandaraja). Our findings are similar to those of a study among 168,212 Asian individuals that noted the superior performance of the Chen formula (ICC: 0.977) compared to the Friedewald and Anandaraja formulas (ICC: 0.975 and 0.901, respectively).[14] However, a previous study in Iran recommended not to use Chen and Anandaraja formulas, particularly in patients with high TG, HDL-C, TC, and FBS as the Anandaraja formula overestimates and underestimates LDL-C in TG levels <300 and >300 respectively, and Chen formula overestimates LDL-C in all

Table 3. Sensitivity and Specificity of Formulas in LDL-C Calculation (using an LDL-C cut-off of 100 mg/dL) in Different TG Subgroups

		Possible, Probable, or Definite			Probable or Definite FH		
TG		**N**	**Sensitivity (%)**	**Specificity (%)**	**N**	**Sensitivity (%)**	**Specificity (%)**
Total	LDL-C, Friedewald (mg/dL)	1148	95.5	89.4	279	98.1	79.0
	LDL-C, Anandaraja (mg/dL)		95.7	81.1		98.1	70.9
	LDL-C, Chen (mg/dL)		97.9	89.5		98.6	74.2
51–150 (mg/dL)	LDL-C, Friedewald (mg/dL)	599	98.1	86.9	132	98.9	73.5
	LDL-C, Anandaraja (mg/dL)		98.5	76.7		100	67.6
	LDL-C, Chen (mg/dL)		98.5	89.8		98.8	76.5
151–200 (mg/dL)	LDL-C, Friedewald (mg/dL)	281	96.7	92.7	70	100	86.7
	LDL-C, Anandaraja (mg/dL)		94.6	84.4		98.2	66.7
	LDL-C, Chen (mg/dL)		98.4	88.5		100	60.0
>200 (mg/dL)	LDL-C, Friedewald (mg/dL)	259	91.4	96.8	73	96.7	91.7
	LDL-C, Anandaraja (mg/dL)		92.4	96.7		95.1	91.7
	LDL-C, Chen (mg/dL)		96.4	88.7		96.7	83.3

Data are shown as the percentage of sensitivity and specificity.
TG, triglycerides; LDL-C, low-density lipoprotein; FH, familial hypercholesterolemia.

Table 4. Correlation and Intra-Class Correlation Coefficient Between Measured and Calculated LDL

		Possible, probable, or definite				Probable or definite			
TG		***r****	***P***	**ICC (95% CI)****	***P***	***r****	***P***	**ICC (95% CI)****	***P***
Total	LDL-C, Friedewald (mg/dL)	0.950	<.001	0.936 (0.929, 0.943)	<.001	0.930	<.001	0.918 (0.880, 0.941)	<.001
	LDL-C, Anandaraja (mg/dL)	0.928	<.001	0.917 (0.902, 0.929)	<.001	0.916	<.001	0.905 (0.860, 0.933)	<.001
	LDL-C, Chen (mg/dL)	0.962	<.001	0.958 (0.944, 0.967)	<.001	0.939	<.001	0.930 (0.891, 0.953)	<.001
51–150 (mg/dL)	LDL-C, Friedewald (mg/dL)	0.963	<.001	0.951 (0.925, 0.966)	<.001	0.936	<.001	0.902 (0.722, 0.953)	<.001
	LDL-C, Anandaraja (mg/dL)	0.945	<.001	0.922 (0.803, 0.959)	<.001	0.933	<.001	0.916 (0.810, 0.955)	<.001
	LDL-C, Chen (mg/dL)	0.965	<.001	0.961 (0.948, 0.971)	<.001	0.938	<.001	0.929 (0.886, 0.954)	<.001
151–200 (mg/dL)	LDL-C, Friedewald (mg/dL)	0.966	<.001	0.948 (0.935, 0.959)	<.001	0.929	<.001	0.907 (0.839, 0.945)	<.001
	LDL-C, Anandaraja (mg/dL)	0.957	<.001	0.947 (0.934, 0.958)	<.001	0.917	<.001	0.917 (0.861, 0.950)	<.001
	LDL-C, Chen (mg/dL)	0.967	<.001	0.962 (0.945, 0.973)	<.001	0.931	<.001	0.923 (0.865, 0.954)	<.001
>200 (mg/dL)	LDL-C, Friedewald (mg/dL)	0.938	<.001	0.890 (0.857, 0.915)	<.001	0.949	<.001	0.913 (0.866, 0.945)	<.001
	LDL-C, Anandaraja (mg/dL)	0.936	<.001	0.880 (0.836, 0.911)	<.001	0.946	<.001	0.923 (0.880, 0.951)	<.001
	LDL-C, Chen (mg/dL)	0.945	<.001	0.937 (0.913, 0.953)	<.001	0.952	<.001	0.940 (0.938, 0.983)	<.001

LDL-C, low-density lipoprotein; ICC, intra-class correlation coefficient. (*)<0.05; (**)<0.01

TG levels.[15] Another study that compared the performance of 11 formulas in Iran pointed out that Friedewald and Chen Formulas had the closest results to direct LDL-C. The Friedewald formula showed a better correlation than the Chen formula, which was in contrast to our results.[16] In a study on the Indian population, Krishnaveni et al. suggested that Anandaraja and Friedewald formulas correlated maximally with direct measurement in TG levels below 100 and above 100, respectively.[17]

Our data also suggest that although the Friedewald formula has a good correlation with the direct method in TG levels above 200, it does not perform as well as the other 2 formulas. It should be noted that the Friedewald formula is known to have a debatable accuracy in TG levels above 200 and is invalid in TG levels above 400.[18] Miller et al[9] found high concordance between the Friedewald formula and the direct method in the total population and FH patients ($r=0.96$ and 0.77, respectively). There is no data regarding the performance of Chen and Anandaraja formulas in the FH population. Our findings propose that LDL-C levels in FH patients can be calculated by both Chen and Friedewald formulas, especially in high TG concentrations. One of the most important limitations of this study is that the reference method which is ultracentrifugation was not used for LDL-C measurement, and LDL-C was measured only using 1 direct method. LDL-C levels are not corrected for Lipoprotein (a), leading to overestimation of LDL-C since FH patients have high Lipoprotein (a) concentrations too.[19] Only 3 common formulas are compared in this study, and future research needs to be conducted to evaluate the other formulas in the FH population, particularly the novel 180-cell method.[20] Target achievement after therapy can also be calculated using LDL-C formulas in future studies.

Table 5. Frequency (%) of Probable or Definite FH for each LDL Definition and Kappa Values for Agreement Between Measured LDL-C and Different Definitions

	N (%)	Kappa ± SE
LDL-C, Friedewald (mg/dL)	299 (26.0)	0.880 ± 0.016
LDL-C, Anandaraja (mg/dL)	307 (26.7)	0.849 ± 0.018
LDL-C, Chen (mg/dL)	291 (25.3)	0.888 ± 0.016
LDL-C, measured(mg/dL)	279 (24.3)	

LDL-C, low-density lipoprotein; SE, standard error.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Isfahan University of Medical Sciences (Approval Date: June 2020; Approval Number: 297182).

Informed Consent: Informed consent was obtained from all participants who participated in this study.

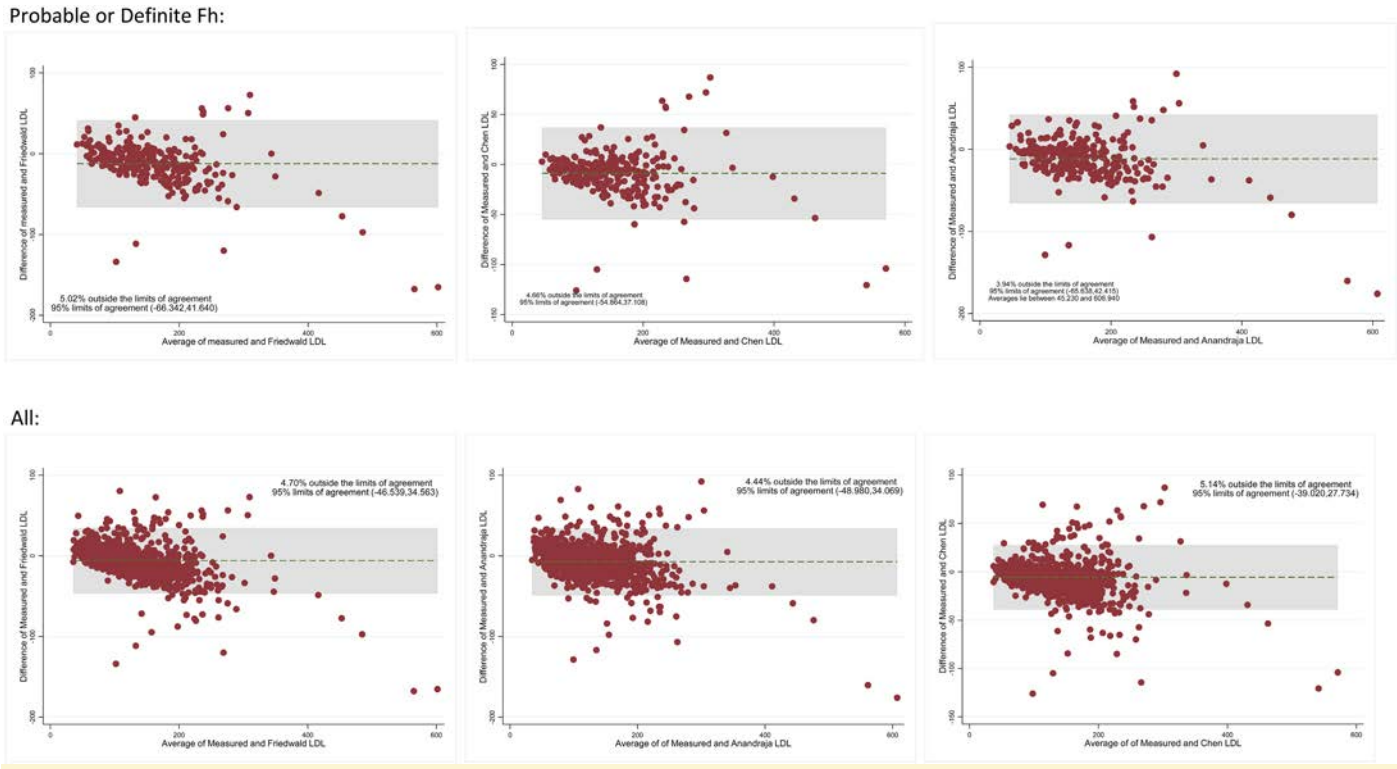

Figure 1. In the Bland–Altman plots the difference between the direct LDL-C and the calculated LDL-C by the 3 formulas in FH patients is plotted against the average of the methods.

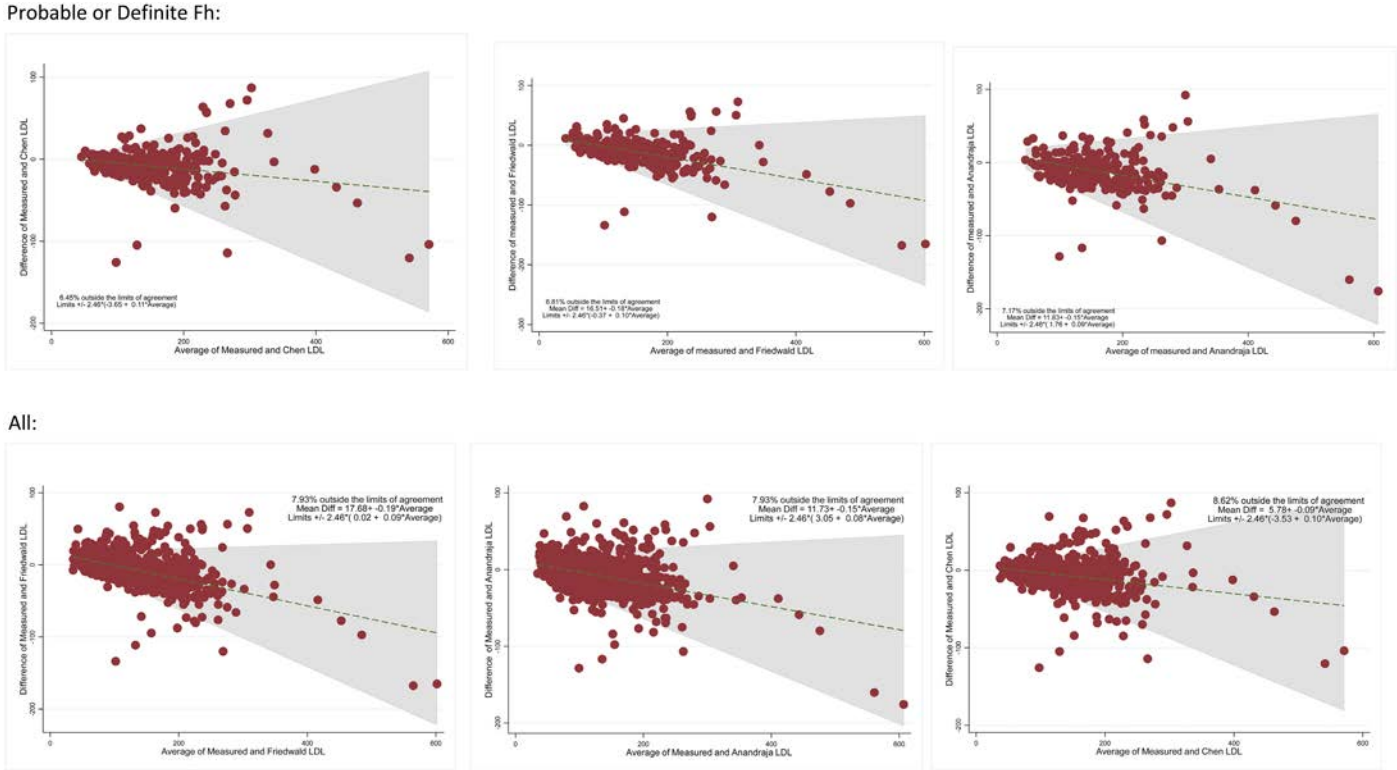

Figure 2. In the Bland–Altman plots the difference between the direct LDL-C and the calculated LDL-C by the 3 formulas in FH patients is plotted against the average of the methods. Trends have also been considered.

Peer-review: Externally peer-reviewed.

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