Case Report

The Case of Negative Low Density Lipoprotein Cholesterol

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Abstract

Accurate estimation of low-density lipoprotein cholesterol (LDL-C) is critical for lipid assessment and cardiovascular risk stratification, yet conventional calculation methods may fail in specific clinical contexts in pediatric population. The Friedewald equation, long considered standard, is unreliable in moderately elevated TG (TG ≥400 mg/dL) and in low total cholesterol (TC) scenarios. Alternative equations, including Martin–Hopkins and Sampson–NIH, have been developed to improve accuracy, but pediatric validation remains limited. We present two pediatric cases highlighting these challenges. Case 1 involved a 5-year-old male with perinatally acquired HIV/AIDS, low TC, and moderately elevated triglyceride (TG) <300 mg/dL. Friedewald-calculated LDL-C was negative, while direct beta-quantification revealed LDL-C of 36 mg/dL and Martin–Hopkins equation underestimated LDL-C by 70%. Case 2 involved a female with type 2 diabetes and high-risk B-cell acute lymphoblastic leukemia, with extreme hypertriglyceridemia >1000 mg/dL and elevated TC. In this case, both Friedewald and Martin–Hopkins calculations failed, whereas Sampson–NIH 2025 provided a calculable LDL-C of 26 mg/dL. These cases demonstrate that TG-to-TC ratios, rather than TG alone, predict calculation failure, and that alternative equations, particularly Sampson–NIH 2025, offer improved reliability. These findings are especially relevant for patients receiving cholesterol-lowering therapies, in whom low LDL-C and elevated TG may lead to significant underestimation of residual atherogenic risk. Further studies using comparative beta-quantification are needed to identify the most accurate LDL-C estimation methods across diverse pediatric populations and low-cholesterol states.

Introduction

Since its introduction in 1972, the Friedewald equation has served as a cornerstone of lipid assessment, providing a rapid and inexpensive estimate of low-density lipoprotein cholesterol (LDL-C) using the formula LDL-C = TC − HDL-C − TG/5 [1]. This calculation relies on several key assumptions, that very-low-density lipoprotein cholesterol (VLDL-C) represents approximately 20% of plasma triglycerides (TG) [1]. In most individuals, these assumptions hold sufficiently well to allow clinically acceptable LDL-C estimation. However, the Friedewald equation is known to fail when TG levels exceed 400 mg/dL (>4.52 mmol/L), at which point VLDL-C estimation becomes unreliable.

To address these limitations, several alternative equations have been developed [2-7]. The Martin–Hopkins equation, introduced in 2013 and validated primarily in adult populations, uses an adjustable TG: VLDL-C ratio derived from population-based strata using non-HDL-C and TG values [2]. This formula is a modified Friedewald equation with an adjustable factor, as LDL-C = TC − HDL-C − TG/adj factor. This approach provides more individualized VLDL-C estimation, improving LDL-C accuracy, particularly at low LDL-C concentrations (LDL-C <70 mg/dL or <1.8 mmol/L) and TG levels <400 mg/dL [2]. In 2023, an extended Martin-Hopkins equation was published with improved LDL-C accuracy in patients with moderate hypertriglyceridemia with TG even upto 800 mg/dL or 9.0 mmol/L [3]. A large review of 23 equation using >5000,000 patients suggested that this Martin–Hopkins formula remains the most accurate method for LDL-C calculation [4]. However, its clinical implementation by Lab Information Systems is limited by the need for a lookup table (~240 strata) to determine the adjustable factor [4-6].

More recently, in 2025, a modified version of the Sampson-NIH was published in Clinical Chemistry, where LDL-C is calculated as: LDL-C = non-HDL-C - (TG/8.37) - (TG x non-HDL-C/2640) + (TG2/ 17400) [7,8]. This modification builds upon the original equation described by Sampson et al in 2020, in which a multivariable regression-based equation was designed to more accurately model VLDL-C across a wider range of lipid profiles, including high triglycerides and low LDL-C, and was validated for triglyceride levels up to 800 mg/dL, outperforming theFriedewald equation in these setting [7,8]. The 2025 Sampson-NIH formula derived and validated in cohorts that included individuals with triglyceride levels exceeding 800 mg/d, reduces systematic underestimation at LDL-C levels <70 mg/dL (1.8 mmol/L) and showed closer agreement with beta-quantification, making it well suited for patients receiving modern lipid-lowering therapies [8,9]. In this report, we present two pediatric cases in which the Friedewald and Martin-Hopkins equations failed to provide a calculated LDL-C values, highlighting the limitations.

Case presentation

Case 1

A 5-year-old African American male with perinatally acquired HIV infection, currently with AIDS due to poor adherence to antiretroviral therapy, underwent routine lipid screening. Laboratory evaluation identified markedly low TC of 59 mg/dL, moderately elevated TG of 272 mg/dL, and low HDL-C of 11 mg/dL. LDL-C calculated using the Friedewald equation yielded a negative/incalculable value. Subsequent beta-quantification via ultracentrifugation revealed an LDL-C of 36 mg/dL, below the reference range but clearly quantifiable. Calculated LDL-C values using alternative equations demonstrated substantial negative bias: 16 mg/dL by the Martin–Hopkins equation, underestimating measured LDL-C by approximately 50–70% (Table 1)

Case 2

A female patient with type 2 diabetes mellitus and high risk B-cell acute lymphoblastic leukemia (HR B-ALL), undergoing chemotherapy complicated by severe hypertriglyceridemia and disc herniation, had extreme lipid derangements. Lipid studies revealed extreme hypertriglyceridemia (TG 1435 mg/dL), elevated total cholesterol (TC 221 mg/dL), HDL-C of 48 mg/dL, and non-HDL cholesterol of 173 mg/dL. Due to the extreme hypertriglyceridemia, LDL-C could not be calculated using the Friedewald or Martin–Hopkins equations; however, the Sampson–NIH 2025 equation yielded a calculated LDL-C of 26 mg/dL. (Table 1). No direct LDL-C assay was performed, and beta quantification was not available due to insufficient sample volume.

Table 1: Lipid parameters, TG/TC ratio, measured LDL-C (beta-quantification), and calculated LDL-C values using 3 different equations in both cases.

	TG	TC	HDL	Non-HDL (calculated)	TG/TC ratio	Beta-quant LDL	Calculated LDL (Friedewald equation)	Calculated LDL (Martin-Hopkins 2023)	Calculated LDL (modified Sampson-NIH 2025)
Ref Range	45-203 mg/dL	112-208 mg/dL	35-73 mg/dL	<145 mg/dL		60-140 mg/dL			
Case 1	272	59	11	48	4.6	36	-6 (Not calculable)	16	15
Case 2	1435	221	48	173	6.4	na	-114 (Not calculable)	-4 (Not calculable)	26

TG: Triglycerides; TC: Total cholesterol; HDL: High-density lipoprotein cholesterol; Non-HDL: Total cholesterol minus HDL; Beta-quant LDL-C: LDL cholesterol measured by ultracentrifugation; na: not available

Discussion

These two pediatric cases illustrate the limitations of conventional LDL-C estimation methods in extreme or atypical lipid contexts. Case 1 demonstrates that low total cholesterol combined with moderately elevated triglycerides can produce negative or non-calculable LDL-C values using the Friedewald equation, even when TG levels are well below the classical 400 mg/dL threshold. Alternative formulas, including the Martin–Hopkins and Sampson–NIH equations yielded calculable LDL-C values, but all substantially underestimated LDL-C compared with beta-quantification, with Friedewald showing the most pronounced negative bias. This observation suggests that disproportionately low TC, rather than moderately elevated TG alone, can precipitate calculation failure. Instability occurs because the fixed TG ÷ 5 assumption overestimates VLDL-C when triglycerides are disproportionately high relative to total cholesterol. As the triglyceride-to-total cholesterol (TG/TC) ratio approaches 5, the likelihood of equation failure increases; in Case 1, the TG/TC ratio is 4.6, placing it near this instability threshold (Table 1). From a pathophysiologic standpoint, poorly controlled HIV and AIDS contribute to impaired hepatic lipoprotein synthesis, increased VLDL-TG secretion, and reduced lipoprotein lipase activity, resulting in low LDL-C and moderately elevated TG [10-12]. Together, these mechanisms disrupt the expected relationship between TG and TC, underscoring that the TG–TC balance, rather than absolute TG alone, is central to understanding equation performance and limitations (Table 2). If validated in larger and more diverse pediatric cohorts, the TG/TC ratio may serve as an adjunctive laboratory flag to prompt reflex direct LDL-C measurement, apolipoprotein B testing, or preferential reporting of non-HDL-C.

Case 2 underscores the impact of extreme hypertriglyceridemia on LDL-C estimation. TG levels exceeding 1400 mg/dL, likely driven by asparaginase therapy and insulin resistance, disrupted the TG-TC relationship, rendering the Friedewald and Martin–Hopkins equations non-calculable. In contrast, the Sampson–NIH 2025 formula produced a calculable LDL-C value, demonstrating improved equation stability in extreme lipid perturbations. However, in the absence of beta-quantification, the analytical accuracy of this estimate cannot be determined. A key observation from both cases is that TG/TC ratios exceeding 4.6 (Case 1) and 6.4 (Case 2) were associated with calculation instability in the Friedewald and Martin-Hopkins equations respectively (Table 2). Relying solely on calculated LDL-C in these contexts risks underestimating LDL particle burden and misclassifying lipid profiles, particularly in pediatric and medically complex populations [9,13]. While the modified Sampson–NIH (2025) equation appears more resilient in calculating a LDL value at high TG and low TC setting, caution is still warranted at extreme lipid perturbations. Direct measurement by Beta-quantification, or assessment of apolipoprotein B remains the gold standard for accurate LDL-C evaluation under such extreme physiological conditions. In summary, these cases highlight: (1) Friedewald LDL-C calculation can fail in the setting of low TC even if TG <300 mg/dL producing negative or severely underestimated values. Elevated TG/TC ratios rather than absolute TG alone may be associated with increased likelihood of LDL-C calculation failure. (2) In high TG scenario, alternative equations, including Martin–Hopkins and modified Sampson–NIH (2025) equation, may generate calculable LDL-C value but may not fully eliminate bias, and Martin equation fails to obtain a calculable value at TG/TC>6.4. (3) Direct LDL-C measurement, apolipoprotein B or non-HDL-C markers remain the benchmark in pediatric patients with atypical lipid profiles, systemic illness or complex metabolic states. Clinicians should interpret calculated LDL-C with caution, considering TG/TC ratios and underlying physiologic context.

Table 2: Predictors of LDL-C calculation reliability in extreme lipid contexts.

Situation	Equations reliability to calculate LDL
1. TG ≥ 400 (classic cutoff) 2. Suggested TG/TC > 4.6 (even if TG <400)	Friedewald incalculable Most equations underestimate LDL-C at high TG/TC ratio
1. TG>800 2. Suggested TG/TC > 6.4	Martin incalculable Sampson–NIH 2025 provides calculable LDL-C

Conclusion

Case 1 challenges the conventional view that Friedewald LDL-C calculation fails only in extreme hypertriglyceridemia. Our findings suggest that the TG/TC ratio may represent a potential marker of increased likelihood of calculation failure and requires further validation. Even with TG below 400 mg/dL, low TC combined with moderately high triglycerides (>200 mg/dL) can produce severe negative value with Friedewald calculations, demonstrating that low total cholesterol, rather than hypertriglyceridemia alone, can produce calculation failure. Inthis setting, alternative equations, including Martin–Hopkins and Sampson–NIH 2025, generated calculable values but produced negative bias compared to measured beta-quantification. Clinically, such underestimation may obscure residual atherogenic LDL, including small dense particles, and lead to misclassification of lipid status. At very high TG levels, Martin–Hopkins may also become non-calculable, whereas the Sampson–NIH 2025 equation maintains calculability, although accuracy could not be confirmed, as observed in Case 2. Direct homogeneous LDL-C assays represent an alternative approach;however, recent data suggest potential positive

bias at low LDL-C concentrations when compared with beta-quantification (14). Therefore, in high TG/TC scenarios, the modified Sampson–NIH 2025 equation may provide a practical approach when direct measurement is unavailable, while non-HDL-C remains a robust TG-independent marker of atherogenic burden. Clinicians should therefore interpret low calculated LDL-C with caution in the setting of elevated TG and low TC, especially in patients receiving cholesterol-lowering therapies, where calculated LDL-C values may substantially underestimate true atherogenic burden. Further validation in larger pediatric cohorts with extreme lipid states is ongoing.

Author Statement

Dr. Earnest JP Daniel & Dr. Sridevi Devaraj contributed to the conceptualization and design of the case study, interpretation of results, and provided critical revisions to manuscript. Dr. Sridevi Devaraj, an expert in cardiovascular research is the corresponding author providing expert insight into the LDL related findings and relevant clinical implications.

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Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon request. The dataset includes clinical records, laboratory results, and other relevant data used in the manuscript

Artificial Intelligence (AI) usage statement

No AI tools were used in the data analysis or writing of this case study. Clinical data were obtained using EPIC Beaker tools. All interpretations and conclusions were made by the authors in accordance with ethical standards, and no automated pattern-finding tools were employed for analysis or case report writing. AI tools were used solely for grammar and language review.

Ethics Statement

This case report was conducted in accordance with institutional ethical guidelines.

Conflict of Interest

The authors declare no conflicts of interest relevant to this work.

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