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Modern Low-Density Lipoprotein Cholesterol Formulas Outperform Direct Methods in Patients with Hypertriglyceridemia and Low Levels of Low-Density Lipoprotein Cholesterol

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Guest: Dr. Jing Cao from the University of Texas Southwestern Medical Center and Children's Health, Dallas.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett. Low-density lipoprotein cholesterol, or LDL, plays a predominant role in the estimation of atherosclerotic cardiovascular disease risk. Its use is firmly cemented in clinical guidelines from several professional societies, with specific treatment or other intervention recommended at different LDL concentration thresholds. For convenience, clinical labs typically calculate LDL, with the Friedewald equation being the most popular choice. Despite its popularity, Friedewald has several notable limitations, including inaccuracies at high triglyceride concentrations or low LDL concentrations. In these scenarios, labs reflex to direct LDL assays for an accurate measurement. Recent years have witnessed the publication of several new LDL calculation formulas that show better performance at much higher triglyceride concentrations. How do these formulas compare to direct methods? And is it possible they'll replace direct methods altogether?

A new research article, appearing in the November 2025 issue of *Clinical Chemistry*, evaluates direct LDL assays and several calculation equations to determine which approach agrees best with the gold standard reference method. In this podcast, we welcome the article's corresponding author. Dr. Jing Cao is a board-certified clinical chemist, Associate Professor of Pathology at the University of Texas Southwestern Medical Center, and Director of Chemistry, Metabolics, and Point-of-Care Testing at Children's Hospital, Dallas. So, Dr. Cao, what motivated your team to compare direct LDL-C assays with modern calculation methods in patients with hypertriglyceridemia and low LDL-C?

Jing Cao: Thank you for having me. Our motivation came from a recurring issue we observed in clinical practice at Mayo Clinic and at the University of Texas Southwestern, the lack of consistency and accuracy in LDL-C results when triglycerides are elevated or LDL-C is low. In such cases, the traditional Friedewald calculation, which is still being used in many laboratories, tends to underestimate LDL-C. At the same

time, we noticed that direct LDL-C assays, which are often assumed to be more accurate, can also perform poorly in dyslipidemic samples. This has become more prominent after we adopted modern calculations that we found inconsistent results between calculation and direct method. These gaps motivated us to systematically evaluate newer LDL-C estimation methods against direct assays, particularly in the patient population most prone to these discrepancies.

Bob Barrett: That's interesting. Were there specific clinical challenges or gaps in guideline recommendations that prompted this study?

Jing Cao: The measurement of direct LDL-C is far from standardized. Different laboratories use different direct assays and calibration systems leading to variability. Existing guidelines haven't kept pace with these analytical realities. Those from the cardiology societies continue to suggest direct testing, but don't recognize that some of the newer calculation methods may outperform direct assays. That disconnect was one of the biggest motivations for our work.

Bob Barrett: Can you explain the different versions of LDL-C calculation, including LDL-C Martin from 2021 and modified Sampson from 2025 that were studied in this article?

Jing Cao: The Friedewald equation, developed in the early 1970s, assumes a fixed triglyceride to VLDL-C ratio of 5, which doesn't hold true across populations or triglyceride ranges. Over time, several groups have refined this approach. The Martin method introduced an adaptive triglyceride to VLDL-C ratio based on a patient's non-HDLC and triglyceride concentrations through a table search approach. The Martin 2021 version updated these adjustable factors using an extended data set with triglycerides up to 800 mg per deciliter, making it suitable for a larger population. The Sampson equation, first proposed in 2020 by Dr. Alan T. Remaley's group at NIH, is based on beta quantification as the reference method. The modified Sampson 2025 version, which was trained with lipid data from the FOURIER clinical trial of the PCSK9 inhibitor, has even better accuracy at very low LDL-C concentrations.

By consolidating several terms, it is also simpler than the original Sampson equation.

Bob Barrett: So why are these methods improvements upon the traditional Friedewald equation?

Jing Cao: The biggest improvement lies in flexibility and data-driven calibration. The newer models leverage large population-based data to capture the nonlinear relationships between triglycerides and LDL particles. In practical terms, this means that these models tailor the estimation based on each

person's lipid profile. As a result, they maintain accuracy even when triglycerides are moderately or markedly elevated, situations where Friedewald tends to break down.

Bob Barrett: Now your study found that LDL-C Sampson outperformed both direct assays and other calculations methods. What makes this more reliable?

Jing Cao: The Sampson method uses beta quantification as the reference method, which minimizes interference from high triglycerides. The modified Sampson 2025 method further enhances accuracy at very low LDL-C concentrations. We know that neurotherapies like PCSK9 inhibitors and combination lipid lowering regimens often reduce LDL-C into very low ranges below 70 or even 55 mg per deciliter. So Sampson method was derived specifically to fit the modern patient population.

Bob Barrett: Are there limitations or some scenarios where LDL-C-Sampson might still fall short?

Jing Cao: Right, no estimation method is perfect. The Sampson equation may still be less accurate in extreme cases of dyslipidemia where triglycerides exceed 800 mg per deciliter. It also assumes accurate input values for total cholesterol, HDLC, and triglycerides. So analytical errors in those measurements will propagate into the calculation. Additionally, while the Sampson method performs well across fasting and non-fasting states, it has not been fully validated in pediatric populations or in patients with certain secondary dyslipidemia, like nephrotic syndrome. So while it's a major advance, it should still be interpreted within the clinical context.

Bob Barrett: Well, finally, Dr. Cao, let's look ahead. What are the implications of these findings for laboratory practice and guidance development? Do you foresee a shift away from direct LDL-C testing in favor of calculated methods?

Jing Cao: Absolutely, the implications are significant. Our results suggest that calculated LDL-C, when deriving using modern equations like Sampson or Martin, can outperform direct assays, not only in accuracy but also in cost effectiveness and standardization. From a laboratory's perspective, this means that instead of reflexing to expensive direct assays whenever triglycerides are elevated, labs can rely on validated calculation methods for most samples. This improves turnaround time and reduces variability between instruments and manufacturers. For guidelines, our study adds to the growing evidence that rather than setting an arbitrary cutoff of high triglycerides to reflex to direct testing, professional societies could recommend modern calculation methods as the default, reserving accurate direct measurement, such as

beta quantification, only for rare or extreme cases. The automated homogeneous direct assay that most labs still use today may retire. That said, changes takes time. Clinical adoption depends on updated guideline endorsements, instrument software updates, and clinicians' education. So what we learned from this journey is that as laboratory medicine involves, it's important to continuously reassess long-standing assumptions.

LDL-C is one of the most frequently ordered lipid parameters, and yet for decades we've relied on methods that don't reflect current knowledge. Our hope is that studies like ours provide the evidence base to accelerate transition and support harmonized, accurate LDL-C reporting across laboratories.

Bob Barrett: That was Dr. Jing Cao from UT Southwestern Medical Center in Dallas, Texas. She wrote a research article in the November 2025 issue of *Clinical Chemistry*, demonstrating the superiority of new LDL calculation formulas over direct methods in patients with low LDL and she's been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.