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ORIGINAL RESEARCH

Assessing the Accuracy of Estimated Lipoprotein(a) Cholesterol and Lipoprotein(a)-Free Low-Density Lipoprotein Cholesterol

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BACKGROUND: Accurate measurement of the cholesterol within lipoprotein(a) (Lp[a]-C) and its contribution to low-density lipoprotein cholesterol (LDL-C) has important implications for risk assessment, diagnosis, and treatment of atherosclerotic cardiovascular disease, as well as in familial hypercholesterolemia. A method for estimating Lp(a)-C from particle number using fixed conversion factors has been proposed (Lp[a]-C from particle number divided by 2.4 for Lp(a) mass, multiplied by 30% for Lp[a]-C). The accuracy of this method, which theoretically can isolate "Lp(a)-free LDL-C," has not been validated.

METHODS AND RESULTS: In 177 875 patients from the VLDbL (Very Large Database of Lipids), we compared estimated Lp(a)-C and Lp(a)-free LDL-C with measured values and quantified absolute and percent error. We compared findings with an analogous data set from the Mayo Clinic Laboratory. Error in estimated Lp(a)-C and Lp(a)-free LDL-C increased with higher Lp(a)-C values. Median error for estimated Lp(a)-C <10 mg/dL was −1.9 mg/dL (interquartile range, −4.0 to 0.2); this error increased linearly, overestimating by +30.8 mg/dL (interquartile range, 26.1–36.5) for estimated Lp(a)-C ≥50 mg/dL. This error relationship persisted after stratification by overall high-density lipoprotein cholesterol and high-density lipoprotein cholesterol subtypes. Similar findings were observed in the Mayo cohort. Absolute error for Lp(a)-free LDL-C was +2.4 (interquartile range, −0.6 to 5.3) for Lp(a)-C<10 mg/dL and −31.8 (interquartile range, −37.8 to −26.5) mg/dL for Lp(a)-C≥50 mg/dL.

CONCLUSIONS: Lp(a)-C estimations using fixed conversion factors overestimated Lp(a)-C and subsequently underestimated Lp(a)-free LDL-C, especially at clinically relevant Lp(a) values. Application of inaccurate Lp(a)-C estimations to correct LDL-C may lead to undertreatment of high-risk patients.

Key Words: LDL-C ■ lipoprotein(a)

Elevated lipoprotein(a) (Lp[a]) is independently associated with atherosclerotic cardiovascular disease (ASCVD) as well as calcific aortic valve stenosis.[1–5] Mendelian randomization studies implicate Lp(a) in cardiovascular morbidity and mortality.[3,6–8] Moreover, it has been shown that patients with high levels of Lp(a) still have higher absolute risk of major adverse cardiac events despite having well-controlled low-density lipoprotein cholesterol (LDL-C) levels.[9,10] The contribution of Lp(a) to atherogenesis elevates it as an important variable to incorporate in cardiovascular risk assessment.

As our understanding of Lp(a) has evolved with respect to risk assessment and diagnosis, so too has

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CLINICAL PERSPECTIVE

What Is New?

- Estimation of the lipoprotein(a) (Lp[a]) cholesterol component, using fixed conversion factors from Lp(a) molar concentration measurements, have arisen but remain inaccurate and overestimate Lp(a) cholesterol, especially at clinically relevant Lp(a) values.
- Application of such inaccurate Lp(a) cholesterol estimations to correct low-density lipoprotein cholesterol risks misinterpretation and underuse of proven low-density lipoprotein cholesterol–lowering therapies in high-risk populations for atherosclerotic cardiovascular disease.

What Are the Clinical Implications?

- If Lp(a) cholesterol components are used in clinical decision-making for atherosclerotic cardiovascular disease risk, we recommend alternatives to estimation with fixed-conversion factors, preferably with approaches that encompass the Lp(a) contribution to total atherogenic particles on a molar scale.

Nonstandard Abbreviations and Acronyms

Lp(a)-C	lipoprotein(a) cholesterol
Lp(a)-P	lipoprotein(a) particle number
VAP	vertical auto profile
VLDbL	Very Large Database of Lipids

the therapeutic landscape. Serum Lp(a) concentrations are almost entirely genetically determined, stable through one's lifetime, and have been resistant to lifestyle changes as well as conventional lipid-lowering therapies[7,11,12] such as statins,[13] estrogen,[14] and fibrates.[12] Fortunately, more effective therapies are emerging. In Europe, apheresis therapy has been used for Lp(a) levels >60 mg/dL.[15] More recently, PCSK9 inhibitors and mipomersen, an apolipoprotein B antisense oligonucleotide, have been found to decrease Lp(a) levels by 25% to 30%.[16–18] Antisense oligonucleotides targeting the production of apolipoprotein(a) (ApoA) have been shown in kinetics studies to correlate with decreased plasma Lp(a) levels.[19] N-acetylgalactosamine-containing antisense oligonucleotides targeting hepatocytes decreased Lp(a) by as much as 50% to 80% in phase II clinical trials, with phase III trials underway.[20,21]

Advancements in risk assessment, diagnosis, and treatment response to current and future therapies have increased the impetus for accurate measurement of the atherogenic burden of Lp(a). Currently, the reporting of Lp(a) levels varies substantially. The International Federation of Clinical Chemistry and Laboratory Medicine recommends Lp(a) particle number (Lp[a]-P), an assessment of molar concentration, as the clinical standard for assessing Lp(a).[22] However, in contemporary clinical cohorts, Lp(a) levels have often been expressed in mass concentration (mg/dL) of the entire lipoprotein particles (eg, cholesterol, esters, phospholipids, triglycerides, and apolipoproteins) to allow familiarity in evaluating cardiovascular risk.[3,23,24]

A proposed method for estimation of Lp(a) cholesterol (Lp[a]-C) has emerged by dividing Lp(a)-P (in nmol/L) by a conversion factor of 2.4 to reflect mass in mg/dL and then multiplying by 30% to reflect the cholesterol content of Lp(a).[22,25–28] This method is of clinical interest since the calculated LDL-C value on a standard lipid panel contains Lp(a)-C. As such, the ability to express Lp(a)-C as a measure of cholesterol content within Lp(a) particles may be appealing to clinicians. Comparing Lp(a)-C with LDL-C would provide clinicians with a qualitative understanding of the degree to which Lp(a) is contributing to the underlying atherosclerotic disease process. Moreover, assessing Lp(a)-C might help to explain situations wherein LDL-C reduction is less than anticipated with LDL-targeted therapies such as statins. There is evidence that statins, by far the most used lipid-lowering therapy, do not lower Lp(a) and in some cases may modestly increase Lp(a) levels.[13,29] Since Lp(a)-C is a component of LDL-C, individuals with high Lp(a)-C levels therefore may have a less-than-expected response to statin therapy. There has also been interest in using Lp(a)-C to isolate the Lp(a)-free LDL-C component, allowing clinicians to arrive at a closer estimation of what is the "real" amount of circulating LDL-C. Because society guidelines employ clinical rather than real LDL-C to guide lipid therapy, we do not advocate for the use of LDL-C correction for Lp(a)-C. However, if inappropriately applied in this way to guide treatment decisions based on established LDL-C targets, the perils of estimated Lp(a)-C inaccuracies may be further amplified.

We used the VLDbL (Very Large Database of Lipids) to assess the accuracy of estimated Lp(a)-C in all patients with measured Lp(a)-C by vertical auto profile (VAP) ultracentrifugation. Because of theoretical concerns that VAP ultracentrifugation can misestimate Lp(a)-C by failing to differentiate Lp(a)-C from buoyant subtypes of high-density lipoprotein (HDL), we included data from a database at the Mayo Clinic employing a separate methodology to directly measure Lp(a) mass via electrophoresis. We hypothesized that if errors related to Lp(a)-C estimations were in fact attributable to the use of fixed conversion factors (both 2.4 to convert

particle number to mass, and 30% to convert mass to cholesterol content), then we would identify errors in estimated Lp(a)-C regardless of the methodology used.

METHODS

Study Populations

We identified all patients from the VLDbL with measured Lp(a)-P and Lp(a)-C values. The VLDbL has been previously described in detail, and was declared exempt by the Johns Hopkins institutional review board for informed consent given the use of deidentified data.[30] A total of 177 875 patients from the VLDbL met inclusion criteria. The median age was 57 years (interquartile range [IQR], 45–68 years) with 58% women (see Table 1 for details). In a separate cohort, we identified a subset of 322 patients within the Mayo Clinic with measured Lp(a) mass and Lp(a)-C mass values. Study approval was issued by the Mayo Foundation and Olmsted Medical Center, and informed consent was obtained from all patients in the cohort.

The VLDbL includes whole-particle molar concentration of Lp(a) (Lp[a]-P, nmol/L) measured using the Denka immunoassay. This assay has been shown to correlate with isoform-insensitive ELISA tests, with the institution of the robust method of 5-point assay calibration using reference material traceable to a standardized source from the World Health Organization/International Federation of Clinical Chemistry and Laboratory Medicine (WHO/IFCC).[31,32] A complete cholesterol profile, including "real," or directly-measured LDL-C, intermediate-density lipoprotein cholesterol, and Lp(a)-C (mg/dL), was measured via the VAP—a rapid ultracentrifugation technique that measures cholesterol content within lipoprotein fractions. The VAP method subjects specimens to ultracentrifugation, separating them into various lipoprotein classes and subclasses (ie, LDL_{1-4}, intermediate-density lipoprotein, Lp[a], very low density $lipoprotein_{1-3}$, and

Table 1. Patient Demographics and Lipid Parameters

	VLDbL (n=177 875)	Mayo Clinic (n=322)
Age, y, median (IQR)	57 (45–68)	55 (44–65)
Male sex, n (%)	74 149 (42%)	128 (56%)
Lipid values, median (IQR)		
Total cholesterol, mg/dL	198 (169–230)	N/A
HDL-C, mg/dL	52 (43–64)	N/A
Triglycerides, mg/dL	116 (82–168)	N/A
Measured Lp(a)-C, mg/dL	7 (5–11)	5 (3–15)
Lp(a)-P, nmol/L	47 (21–124)	N/A
Lp(a) particle mass, mg/dL	N/A	21 (6–76)
Measured LDL-C, mg/dL	117 (93–144)	N/A
$LDL\text{-}C_F$, mg/dL	115 (91–143)	N/A
$LDL\text{-}C_N$, mg/dL	117 (94–144)	N/A
Measured Lp(a)-C levels by category, n (%)		
<10 mg/dL	123 877 (69.64%)	200 (60.24%)
10 – 19 mg/dL	45 111 (25.36%)	62 (18.67%)
20 – 29 mg/dL	7614 (4.28%)	34 (10.24%)
30 – 39 mg/dL	1088 (0.61%)	15 (4.52%)
40 – 49 mg/dL	163 (0.09%)	11 (3.31%)
≥50 mg/dL	22 (0.01%)	0 (0%)
Measured Lp(a)-free LDL-C levels by category, n (%)		
LDL-C <70 mg/dL	22 311 (12.54%)	N/A
LDL-C 70 – 99 mg/dL	49 184 (27.65%)	N/A
LDL-C 100 – 129 mg/dL	52 640 (29.59%)	N/A
LDL-C 130 – 159 mg/dL	33 621 (18.90%)	N/A
LDL-C 160 – 189 mg/dL	13 982 (7.86%)	N/A
LDL-C ≥190 mg/dL	6137 (3.45%)	N/A

HDL-C indicates high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; $LDL\text{-}C_F$, low-density lipoprotein cholesterol as estimated by the Friedewald equation; $LDL\text{-}C_N$, low-density lipoprotein cholesterol as estimated by the Martin-Hopkins equation; Lp(a)-C, lipoprotein(a) cholesterol; Lp(a)-P, lipoprotein(a) particle number; N/A, not available; and VLDbL, Very Large Database of Lipids.

HDL_{2-3}). Subsequently, spectrophotometric absorbance is employed to measure the cholesterol composition within each class.[33] The Mayo Clinic data set includes Lp(a) particle mass measurement (mg/dL) via a separate Denka assay, and Lp(a)-C mass measurement (mg/dL) using the Helena serum protein immunofixation electrophoresis cholesterol profile. The Helena serum protein immunofixation electrophoresis assay employs gel electrophoresis to separate lipoprotein classes. Electrophoretic bands are subsequently stained with an enzymatic reagent to quantify the cholesterol composition of each class using densitometric scanning. It has previously been shown using the automated serum protein immunofixation electrophoresis assay that Lp(a) reliably separates from other lipoproteins with minimal interference and the identity of Lp(a)-C containing bands can be confirmed via Western blot targeting ApoA.[34] Ultracentrifugation and electrophoresis are validated methods for separating lipoproteins, and the accuracy of both VAP and serum protein immunofixation electrophoresis methodologies is supported by correlations with reference standards.[33,35]

Statistical Analysis

To derive estimated Lp(a)-C in mg/dL, Lp(a)-P values were divided by 2.4, then multiplied by 0.3 to derive the cholesterol component. In the Mayo Clinic data set, Lp(a) particle mass measurements were multiplied by 0.3 to estimate the cholesterol component.

Absolute and percent error of estimated Lp(a)-C were calculated as compared with measured values (by VAP ultracentrifugation) and stratified by Lp(a)-C level categories. Error was calculated by subtracting measured values from estimated; by extension, positive values represented overestimation, and negative values represented underestimation. Concordance (ie, accuracy) in Lp(a)-C was defined as the percentage of patients with measured Lp(a)-C falling into the same category as estimated Lp(a)-C.

Within the VLDbL, we further assessed accuracy in estimated Lp(a)-free LDL-C after accounting for estimated Lp(a)-C, as estimated LDL-C includes Lp(a)-C. To do this, estimated Lp(a)-free LDL-C was calculated by subtracting estimated Lp(a)-C from LDL-C as calculated by the Martin-Hopkins and Friedewald equations. Estimated Lp(a)-free LDL-C was compared with measured values by VAP of its equivalent respective components, ie, the sum of directly measured LDL-C and intermediate-density lipoprotein cholesterol, referred to as VAP Lp(a)-free LDL-C (see Table 2 for definitions). As with estimated Lp(a)-C, absolute and percent error in estimated versus measured Lp(a)-free LDL-C were calculated and stratified by Lp(a)-C category. We also stratified analysis of both estimated Lp(a)-C and estimated Lp(a)-free LDL-C by overall HDL cholesterol (HDL-C) values and HDL subtypes given prior concern that high levels of HDL-C may lead to inaccuracies in the VAP measurement of Lp(a)-C.[36] Statistical analysis was performed with Stata version 16.0.

RESULTS

With each subsequent increase in estimated Lp(a)-C category, there was a rise in magnitude of error between estimated values and measured values (Figure 1). The median error in the estimated Lp(a)-C category of <10 mg/dL was −1.9 mg/dL (IQR, −4.0 to 0.2), while that in the largest category of Lp(a)-C ≥50 mg/dL was +30.8 mg/dL (IQR, 26.1 to 36.5). As absolute error was calculated by subtracting measured Lp(a)-C (by VAP) from estimated Lp(a)-C, Lp(a)-C was overestimated on average in all categories >10 mg/dL. Further, there was a positive association with estimated Lp(a)-C category and degree of overestimation. Median percent error of estimated Lp(a)-C ranged from −38.5% (IQR, −64.6 to 4.1) for Lp(a)-C <10 mg/dL, to +62% (IQR, 26.6–111.2) for Lp(a)-C between 10 and 19 mg/dL, to >100% (IQR, 72.8–134.3) for Lp(a)-C >40 mg/dL.

In the VLDbL, concordance between estimated Lp(a)-C and VAP Lp(a)-C was 89.3% in patients with Lp(a)-C values <10 mg/dL; concordance decreased, however, for Lp(a)-C beyond 10 mg/dL, to ≤10% at Lp(a)-C categories of 20 mg/dL and beyond (Figure 2, Table S1). In the Mayo clinic database, concordance between estimated Lp(a)-C and measured Lp(a)-C was 99% in patients with Lp(a)-C values <10 mg/dL; concordance decreased, however, for Lp(a)-C beyond 10 mg/dL, to ≤10% at Lp(a)-C categories of 40 mg/dL and beyond.

Table 2. Key Terms Defined

	Estimated	Measured
Lp(a)-C	Lp(a)-P÷2.4=total mass in mg/dL Total mass of Lp(a)×30%=cholesterol component	By VAP ultracentrifugation (abbreviated VAP Lp[a]-C) or densitometric scanning
LDL-C (“clinical”)	By Friedewald or Martin-Hopkins (incorporates cholesterol components of LDL, IDL, and Lp[a])	LDL-Cr*+IDL-C+Lp(a)-C, by VAP (abbreviated VAP LDL-C)
Lp(a)-free LDL-C	LDL-C (by Friedewald or Martin-Hopkins)—estimated Lp(a)-C	LDL-Cr+IDL-C, by VAP (abbreviated VAP Lp[a]-free LDL-C)

IDL-C indicates intermediate-density lipoprotein cholesterol; and Lp(a), lipoprotein(a); and VAP, vertical auto profile.
*LDL-Cr: measured cholesterol component of low-density lipoprotein alone (ie, “real” low-density lipoprotein cholesterol [LDL-C]).

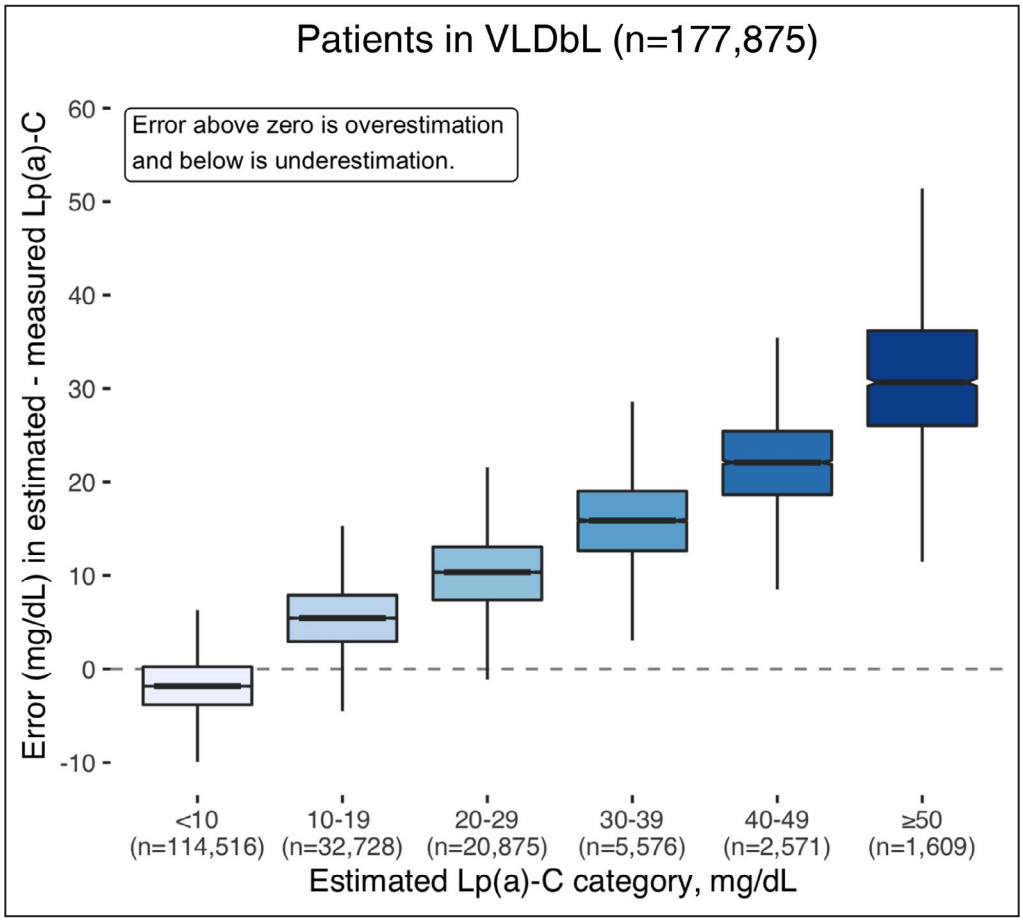

Figure 1. Absolute error in estimated lipoprotein(a) cholesterol (Lp[a]-C), stratified by Lp(a)-C category.
VLDbL indicates Very Large Database of Lipids.

Even in patients with HDL-C <60 mg/dL (where VAP is purported to more accurately measure Lp(a)-C), concordance decreased from >90% at Lp(a)-C <10 mg/dL to <5% at Lp(a)-C of 20 mg/dL and beyond. In patients with HDL-C >60 mg/dL, we observed the same step-off in concordance as estimated Lp(a)-C increased (Figure S1, Table S2). In addition to directly measuring overall HDL-C, VAP further allows for the measurement of HDL-C subtypes. Because of potential concerns that ultracentrifugation techniques may be unable to distinguish between Lp(a) and lipoproteins of comparable density, eg, buoyant HDL_2-C, we performed additional experiments examining whether the degree in error of estimated Lp(a)-C varied among individuals with high and low HDL_2-C. There is a theoretical concern that among individuals with high amounts of HDL_2-C, VAP may misinterpret HDL_2-C as Lp(a)-C and report falsely elevated Lp(a)-C. Error in estimated Lp(a)-C was actually lower among the highest tertile of HDL_2-C, on average by 2 to 3 points (mg/dL) relative to those in the lowest HDL_2-C tertile (Figure S2, Table S3). While this difference could theoretically be explained by contributions of HDL_2-C to Lp(a)-C leading to higher VAP-measured Lp(a)-C and subsequently smaller differences between overestimated Lp(a)-C and measured Lp(a)-C, the difference in error is not meaningful enough to account for the large degree of error observed at clinically meaningful Lp(a)-C values on the order of 20 to 30 points.

In addition, recognizing that varying triglyceride levels also have the potential to further bias the application of the 30% multiplication factor, we also plotted absolute error in estimated Lp(a)-C across ranges of HDL-C, total cholesterol, and triglycerides. In particular, we found that the error in estimated Lp(a)-C continued to increase at clinically meaningful Lp(a)-C values to a similar degree and that ultimately the 30% conversion factor performed poorly across the spectrum of triglyceride levels (Figure 3), as well as a wide range of values for HDL-C and total cholesterol (Figures S3 through S4).

With regard to estimated Lp(a)-free LDL-C, with each subsequent increase in Lp(a)-C category, the degree of error in estimated Lp(a)-free LDL-C became increasingly negative, thereby underestimating Lp(a)-free LDL-C

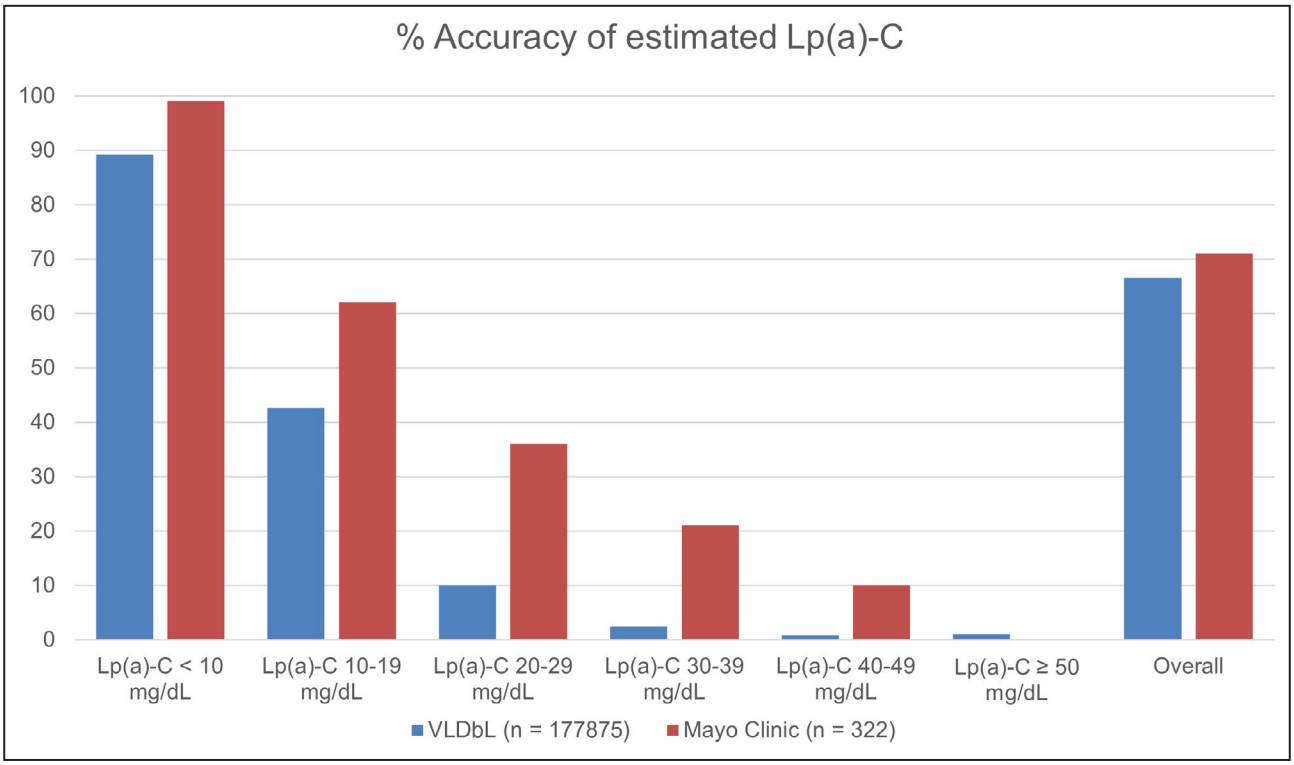

Figure 2. Concordance between estimated lipoprotein(a) cholesterol (Lp[a]-C) and measured Lp(a)-C.
VLDbL indicates Very Large Database of Lipids.

with higher magnitudes (Figure 4). While median error of Lp(a)-free LDL-C (using Martin-Hopkins) was +2.4 mg/dL (IQR, −0.6 to 5.3 mg/dL) in patients with Lp(a)-C <10 mg/dL, the error was −31.8 mg/dL (IQR, −37.8 to −26.5 mg/dL) for patients with Lp(a)-C ≥50 mg/dL. The relationship was similar when calculating LDL-C using the Friedewald equation. Median percent error for estimated Lp(a)-free LDL-C also increased at higher Lp(a)-C categories, from +2.2% (IQR, −0.5 to 5.1) in Lp(a)-C <10 mg/dL, to −30.6% (IQR, −45.2 to −21.5) for Lp(a)-C ≥50 mg/dL (using Martin-Hopkins). Again, a similar relationship in percent error was observed when calculating LDL-C using the Friedewald equation.

DISCUSSION

Lp(a)-C mass concentration estimations are inaccurate at clinically significant Lp(a) molar measurements. We found that estimated Lp(a)-C had substantial inaccuracy, especially at higher values. For estimated Lp(a)-C values >50 mg/dL (with corresponding Lp(a)-P values >400 nmol/L based on the fixed conversion rate), only 1% of those patients had corresponding VAP–Lp(a)-C values >50 mg/dL. These findings were robust to stratification by overall HDL-C concentration and the HDL_2-C subtype, demonstrating that inaccuracy was not attributable to previously described HDL-related inconsistencies with the VAP assay.[36] Furthermore, findings were replicated in the Mayo database, which uses an alternative methodology to directly measure Lp(a)-C, reinforcing that the error is attributable to the use of fixed conversion factors rather than the methodology employed to measure Lp(a)-C.

Lp(a) mass >50 mg/dL is a commonly cited threshold for increased atherogenic outcomes.[15] Applying the conversion factor of 30% between total Lp(a) mass and cholesterol component would imply that, by extension, within the context of previous population studies, Lp(a)-C levels of >15 mg/dL carry increased atherogenic risk. In our data set, we found that nearly all individuals with estimated Lp(a)-C >10 mg/dL, capturing those at this clinically significant threshold and beyond, had significant error when compared with measured values.

Given the degree of inaccuracy, estimations using mass concentration measurements, including Lp(a)-free LDL-C, may be misleading. The overestimation of Lp(a)-C can be problematic when using said estimations to further stratify a patient's LDL-C burden, ie, when calculating measures such as Lp(a)-free LDL-C. The clinical rationale for estimating Lp(a)-free LDL-C lies in the demand for quantifying the contribution of Lp(a)-C within LDL-C estimations by standard lipid panels. Given the inaccuracies in estimating Lp(a)-C using fixed molar-to-mass conversion factors as shown in our study, estimated Lp(a)-free LDL-C had similarly poor correlation with measured VAP Lp(a)-free LDL-C. In addition,

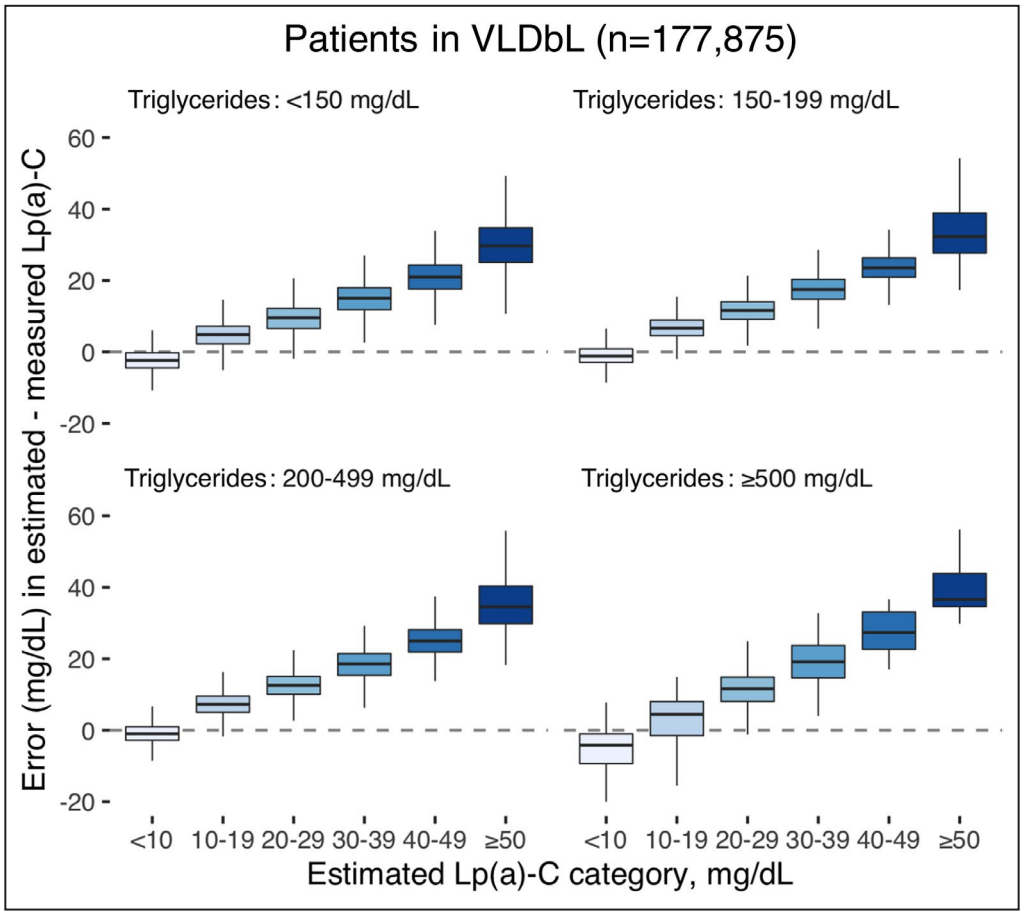

Figure 3. Absolute error in estimated lipoprotein(a) cholesterol (Lp[a]-C), stratified by Lp(a)-C category and triglycerides.
VLDbL indicates Very Large Database of Lipids.

because Lp(a)-C overall was overestimated, by extension, Lp(a)-free LDL-C was consistently underestimated relative to VAP Lp(a)-free LDL-C. As a result, the clinical application of Lp(a)-free LDL-C may underestimate LDL-C and lead to undertreatment. In our data set, we found that individuals with estimated Lp(a)-C >10 mg/dL had underestimated Lp(a)-free LDL-C; these individuals are already at higher risk of ASCVD outcomes given the commonly cited risk threshold for Lp(a) mass of 50 mg/dL (which would correlate to an Lp[a]-C of 15 mg/dL). This is especially notable since the primary mode of treating patients with high Lp(a) is to maximally intensify LDL-lowering therapy, often past guideline-directed targets.[37] Thus, the application of estimated Lp(a)-free LDL-C to clinical LDL-C targets has potential for undertreatment of LDL-C for this high-risk population who should be on maximally aggressive lipid-lowering therapy. Fortunately, existing major society guidelines do not make recommendations to tailor therapies based on this measure, instead focusing on LDL cholesterol alone.

The evidence suggests that measures of molar concentration (Lp(a)-P in nmol/L) most accurately assess the true atherogenic risk of Lp(a). Unlike with mass measurements, there is a widely accepted standardization for Lp(a)-P measurement in place that accounts for the obstacles encountered with attempts at mass estimation.[22,38,1212] This practice is reflected in the widespread use of Lp(a) particle concentration across Europe. However, when using fixed conversation factors between molar and mass concentration, prior obstacles in mass measurement such as heterogeneity in isoform size and cholesterol composition reemerge, which preclude accurate conversion. In Lp(a) mass composition analyses, molar-to-mass conversion rates empirically differed by as much as 1.85 to 2.85, leading to an average of 2.4 to be the proposed conversion factor.[32] This range is likely explained by the isoform variability of ApoA driven by significant variation in the number of kringle 4 (type 2) copies among individuals. Moreover, newer research has demonstrated that the range of cholesterol composition relative to overall Lp(a) mass varies from 6% to 57% among individuals,[28,38] which debunked the 30% figure as derived from original studies isolating Lp(a) and measuring

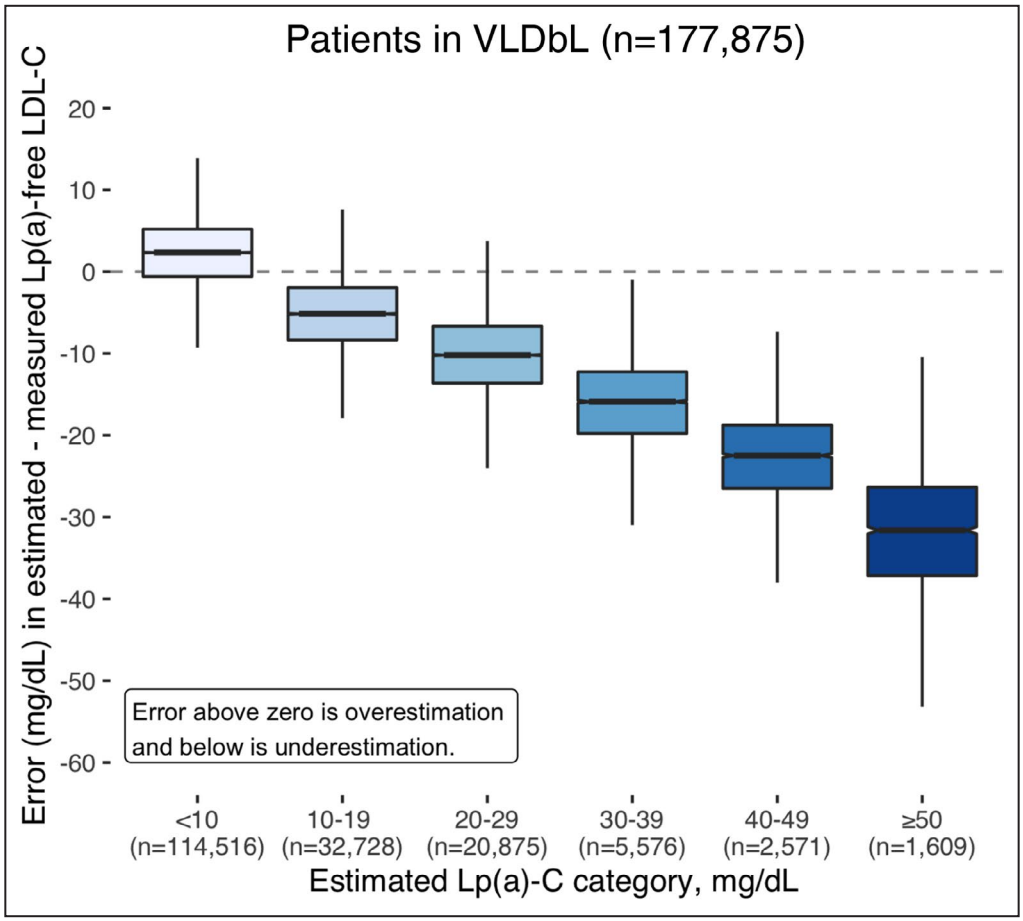

Figure 4. Absolute error between estimated lipoprotein(a) (Lp[a])-free low-density lipoprotein cholesterol (LDL-C) (see Table 2) and vertical auto profile Lp(a)-free LDL-C, stratified by Lp(a) cholesterol (Lp[a]-C) category.
VLDbL indicates Very Large Database of Lipids.

cholesterol content.[26] The wide ranges observed between conversions from particle number to mass and total mass to cholesterol testify to the significant variability in Lp(a) composition both between and within individuals, accentuating the problematic use of single conversion factors for such a complex molecule. Thus, molar-to-mass conversion and accurate estimation of Lp(a)-free LDL require adjustment for variability in all components, including isoform size, cholesterol composition, and even carbohydrate constituents—none of which are implementable for clinical use.

It is important to recognize, however, that commonly used immunoassays to measure Lp(a) particle concentration, including the Denka assay employed in this study, are not in reality isoform-insensitive because they employ antibodies that respond to multiple epitopes. However, gold-standard isoform-insensitive assays are not available for clinical use. As far as commercially available assays go, Denka remains one of the most well-validated owing to its use of a 5-point calibration system to account for a range of ApoA isoform sizes.[39] Because our aim was to demonstrate the clinical implications of Lp(a)-C misestimation, it was considered appropriate to use a clinically and commercially available assay. Nonetheless, clinicians and laboratories should recognize that the accuracy of the Denka assay and other immunoassays rely on appropriate assignment of values to the calibrators that should trace back to the WHO/IFCC reference material.

Nonetheless, we recognize that capturing the relative contribution of Lp(a)-C to LDL-C has real consequences for risk assessment, diagnosis, and treatment, especially in patients with very high risk of ASCVD. Specifically, there may be situations that clinicians may face in which standard lipid-lowering therapy does not reduce cholesterol as expected. In such situations, we propose alternative approaches that may be better suited to clinical use and more reliable than estimation. For example, measurement of Lp(a)-P, where deemed applicable could at least, when elevated, provide a qualitative sense to clinicians that Lp(a) is contributing meaningfully to a patient's

LDL-C. Moreover, investment in and development of assays for direct measurement of Lp(a)-C that are accurate, affordable, and scalable in clinical practice would obviate the need for estimating Lp(a)-C altogether. Reassuringly, promising direct LDL-C assays with high throughput potential are in development.[38] Last, keeping in line with the greater accuracy accompanying molar approaches, a strategy of quantifying total atherogenic particle number using apolipoprotein B and comparing against Lp(a)-P could offer a particle-for-particle illustration of Lp(a)'s contribution to the atherogenic particle load.

Limitations

The VLDbL does not include patient medications or other clinical data, which precludes assessment of pre-treatment or post-treatment status of hyperlipidemia. Specifically, patients who take statins may have increased Lp(a)-P, which may further confound the molar-to-mass conversion ratios used in our study. Furthermore, intrapatient variation in Lp(a)-P was not analyzed; individuals can have 2 separate isoforms, though one usually predominates.[32,40] The VLDbL also does not identify isoforms of ApoA in patients. The data set also could not stratify for certain demographic measures, in particular race and ethnicity.[24,41,42] The Mayo clinic data set could not be stratified by HDL-C because of lack of concurrent standard lipid panel results.

CONCLUSIONS

We found that estimated Lp(a)-C using a series of simple proportionality factors from molar Lp(a) concentration resulted in substantial inaccuracy. Furthermore, our data demonstrate that patients with increased and clinically relevant Lp(a)-P levels (and subsequently, Lp[a]-C estimations) have their Lp(a)-C burden consistently overestimated, which translated to subsequent underestimation of Lp(a)-free LDL-C levels. This bias was especially evident for clinically relevant Lp(a) values and risks underuse of proven LDL-C lowering therapies in high-risk populations for ASCVD. If Lp(a)-C or Lp(a)-free LDL-C are to be used to drive clinical decision-making as it relates to risk assessment, diagnosis, and treatment, we recommend alternatives to the fixed-conversion ratio, such as more qualitative clinical interpretations of Lp(a)-P contribution to ASCVD risk, more widespread direct measurement of Lp(a)-C, or approaches that quantify on a molar scale the Lp(a)-P contribution to total atherogenic particles.

ARTICLE INFORMATION

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Disclosures

Drs Martin and Jones are listed as coinventors on a pending patent filed by Johns Hopkins University for the novel method of low-density lipoprotein cholesterol estimation. Dr Jones has served as an advisor to Sanofi/Regeneron. Dr Jaffe has or presently consults for most of the major diagnostic companies, including Abbott; Ortho; Roche; Siemens; Astellas; Radiometer America, Inc.; Sphingotec; ET Healthcare; Novartis; Amgen; and Beckman-Coulter. Dr Kulkarni was previously an employee at VAP Diagnostics. Dr Santos has received honoraria related to consulting, speaker, and/or research activities from Abbott, Ache, Amgen, Astra Zeneca, EMS, Esperion, Kowa, Libbs, Novo-Nordisk, Merck, Novartis, MSD, Pfizer, PTC Therapeutics, and Sanofi/Regeneron. Dr Toth serves as a consultant for AstraZeneca Amarin Pharma, Inc, and Kowa Company Ltd, and as an expert witness for Amarin Pharma, Inc. Dr Toth has received additional support from Esperion Therapeutics, Inc.; Merck; Novo Nordisk; and Amgen. Dr Martin has served as a consultant in the past 36 months to Amgen, AstraZeneca, Dalcor, Esperion, Kaneka, iHealth, Novo Nordisk, Novartis, Regeneron, REGENXBIO, Sanofi, and 89bio. The remaining authors have no disclosures to report.

Supplemental Material

Tables S1–S3
Figures S1–S4

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Supplemental Material

Table S1. Concordance between estimated Lp(a)-C and measured Lp(a)-C in the VLDbL and Mayo Clinic Database.

	% Accuracy	
Lp(a)-C category	VLDbL	Mayo Clinic
Lp(a)-C < 10 mg/dL	89	99
Lp(a)-C 10-19 mg/dL	42	62
Lp(a)-C 20-29 mg/dL	10	36
Lp(a)-C 30-39 mg/dL	2	21
Lp(a)-C 40-49 mg/dL	1	10
Lp(a)-C ≥ 50 mg/dL	1	0
Overall	66	71

Table S2. Concordance between estimated Lp(a)-C and VAP-Lp(a)-C, stratified by HDL-C category.

	% Accuracy	
Lp(a)-C category	HDL-C ≥ 60	HDL-C < 60
Lp(a)-C < 10 mg/dL	78.0	94.5
Lp(a)-C 10-19 mg/dL	62.4	31.5
Lp(a)-C 20-29 mg/dL	20.7	3.5
Lp(a)-C 30-39 mg/dL	5.2	0.6
Lp(a)-C 40-49 mg/dL	1.7	0.1
Lp(a)-C ≥ 50 mg/dL	1.5	0.6
Overall	62.2	68.8

Table S3. Median error between estimated and directly measured Lp(a)-C (in mg/dL), stratified by HDL-2-C tertile.

Median error between estimated and directly measured Lp(a)-C (in mg/dL), stratified by HDL-2-C tertile

Lp(a)-C category	HDL-2-C < 12 mg/dL (n=70,958)	HDL-2-C 12-16 mg/dL (n=50,988)	HDL-2-C ≥ 17 mg/dL (n=55,929)	Overall (n=177,875)
Lp(a)-C < 10 mg/dL	-1.1	-2.1	-3.7	-1.9
Lp(a)-C 10-19 mg/dL	6.7	5.9	3.6	5.4
Lp(a)-C 20-29 mg/dL	11.7	10.8	8.5	10.3
Lp(a)-C 30-39 mg/dL	17.5	16.4	14.0	15.9
Lp(a)-C 40-49 mg/dL	23.4	23.4	20.2	22.1
Lp(a)-C ≥ 50 mg/dL	32.5	31.7	29.7	30.8
Overall	0.2	0.8	-0.8	0.1

Figure S1. Concordance between estimated Lp(a)-C and VAP-Lp(a)-C, stratified by HDL-C category.

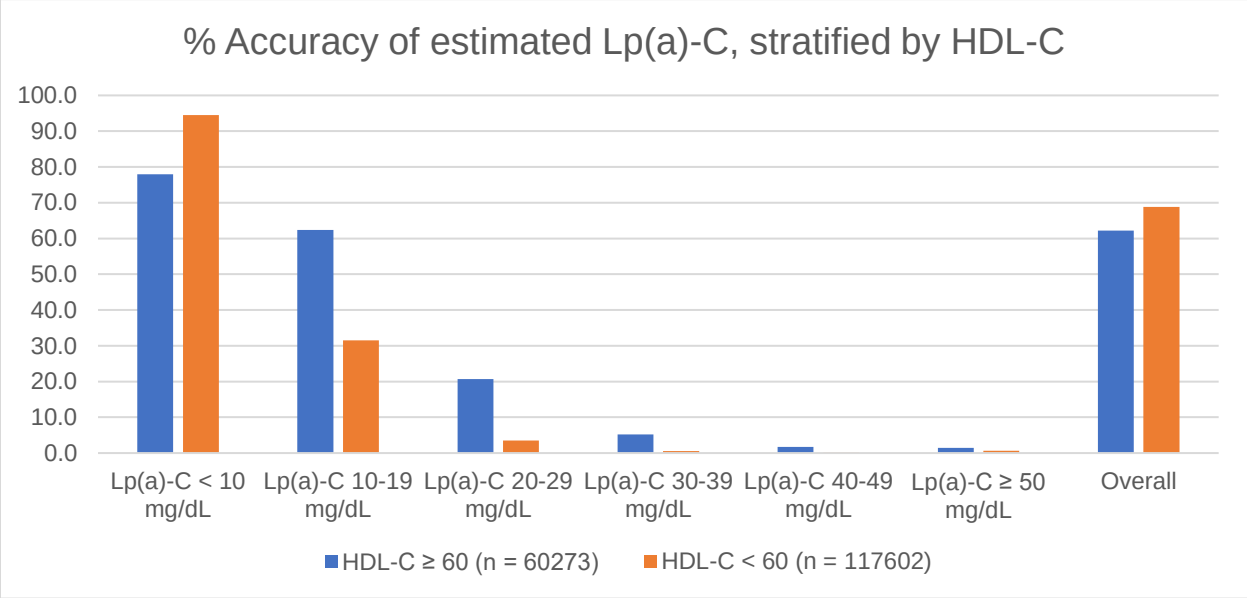

Figure S2. Median error of estimated Lp(a)-C, stratified by HDL-C-2 tertile.

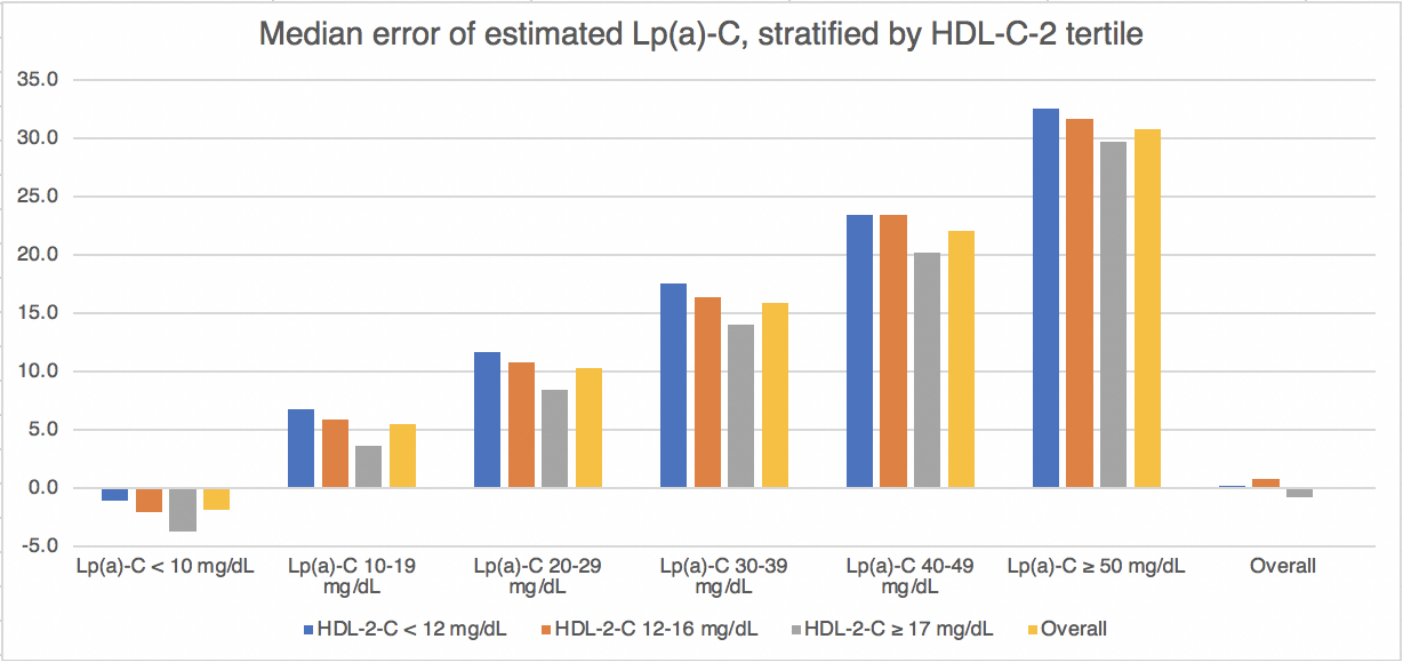

Figure S3. Absolute error in estimated Lp(a)-C, stratified by Lp(a)-C category and HDL-C.

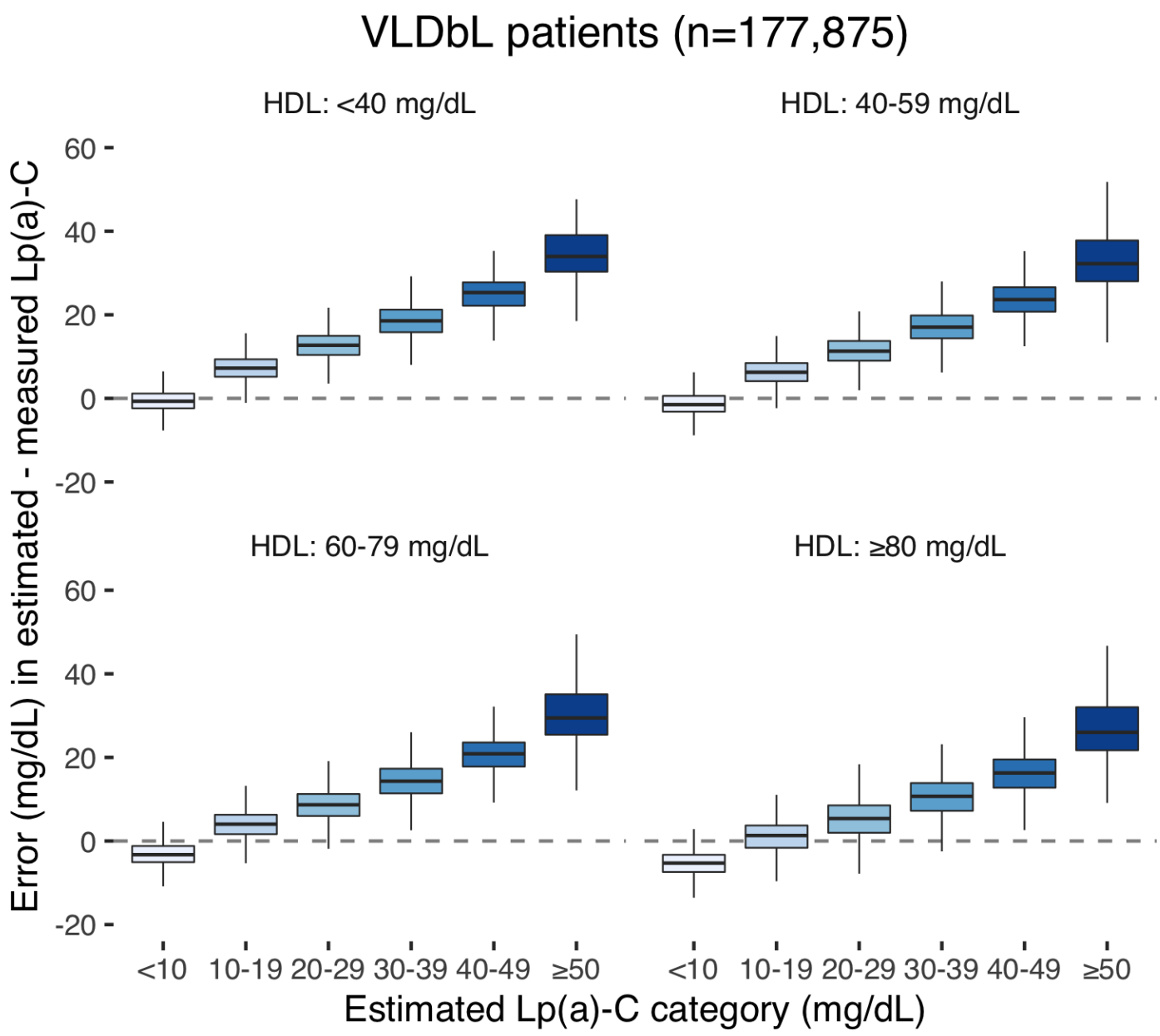

Figure S4. Absolute error in estimated Lp(a)-C, stratified by Lp(a)-C category and Total Cholesterol.