Reducing Lipid Panel Error Allowances to Improve the Accuracy of Cardiovascular Risk Stratification

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BACKGROUND: The standard lipid panel forms the backbone of atherosclerotic cardiovascular disease risk assessment. Suboptimal analytical performance, along with biological variability, could lead to erroneous risk assessment and management decisions. The current National Cholesterol Education Program (NCEP) performance recommendations have remained unchanged for almost 3 decades despite improvements in assay technology. We investigated the potential extent of risk misclassification when the current recommendations are met and explored the impact of improving analytical performance goals.

METHODS: We extracted lipid panel data for 8506 individuals from the NHANES database and used these to classify subjects into 4 risk groups as recommended by the 2018 US Multisociety guidelines. Analytical bias and imprecision, at the allowable limits, as well as biological variability, were introduced to the measured values to determine the impact on misclassification. Bias and imprecision were systematically reduced to determine the degree of improvement that may be achieved.

RESULTS: Using the current performance recommendations, up to 10% of individuals were misclassified into a different risk group. Improving proportional bias by 1%, and fixing imprecision to 3% across all assays reduced misclassifications by up to 10%. The effect of biological variability can be reduced by taking the average of serial sample measurements.

CONCLUSIONS: The current NCEP recommendations for analytical performance of lipid panel assays allow for an unacceptable degree of misclassification, leading to possible mismanagement of cardiovascular disease risk. Iteratively reducing allowable error can improve this.

Introduction

A standard lipid panel evaluation is currently the mainstay of risk assessment and management in both primary and secondary prevention of atherosclerotic cardiovascular disease (ASCVD) (1–3). Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) each have a role in the risk-assessment algorithms recommended in international guidelines on the management of dyslipidemias (1–3). In the US primary prevention algorithm (1), risk assessment begins with a baseline LDL-C determination. If LDL-C is >190 mg/dL (4.9 mmol/L), statin therapy is commenced. An LDL-C of 70 to 190 mg/dL (1.8–4.9 mmol/L) in patients 40 to 75 years old, without diabetes, necessitates a 10-year risk score calculation. This involves applying TC and HDL-C measurements to a pooled cohort equation (PCE). The ensuing risk discussion, and decisions regarding statin therapy, intensity adjustments, and add-on therapies, are informed by appraisal of risk-enhancing factors, including persistently elevated LDL-C and TG (1).

Given their pivotal role in clinical decision-making, errors in the measurement of lipid parameters could lead to errors in risk assessment and the evaluation of response to therapy and thus may result in mismanagement. The current analytical performance specifications, determined in 1990 and 1995 by the National Cholesterol Education Program (NCEP), allow total errors of 8.9%, 13%, and 15% for TC, HDL-C, and TG, respectively (4–7) (Table 1). Calculated LDL-C (cLDL-C), which is currently the primary method to determine LDL-C, is subject to the combined error in these 3 parameters (8, 9). The NCEP recommendations are based on expert opinion and were limited by what was achievable by the state of the art 28 years ago.

The 2015 Milan Hierarchy delineates 3 models for defining analytical performance specifications (10). The

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Table 1. Biological variability from EFLM database.

	Total cholesterol	HDL-C	Triglycerides	LDL-C
Biological variability (EFLM)	5.3	5.8	20	8.3
Allowable imprecision (%)	≤3	≤4 (≥42 mg/dL) (≥1.1 mmol/L)	≤5	≤4
Allowable bias (%)	≤±3	≤±5	≤±5	≤±4
Total error allowable	≤8.9	≤13	≤15	≤12
Proposed imprecision (%)	≤3	≤3 (≥57 mg/dL) (≥1.5 mmol/L)	≤3	
Proposed bias	≤±2	≤±4	≤±4	
Proposed total error (%)	≤8	≤10	≤10	
Scenario				
1		No bias		Variable error
2	Positive bias	Negative bias	Negative bias	Overall positive error
3	Negative bias	Positive bias	Positive bias	Overall negative error

National Cholesterol Education Program and proposed analytical performance recommendations and hypothetical error scenarios.
Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

first bases them on their effect on clinical outcomes, the second bases them on biological variability, and the third bases them on the state of the art. While the first 2 models are considered preferable, any one model may be more applicable to a certain measurand than the others, and a combination of 2 or more models may be appropriate (10). In the case of the lipid panel, the biological variability (BV)-based performance specifications are similar to, or more lenient than, the NCEP-recommended specifications, as these measurands suffer from relatively wide BV (Table 1) (11). As far as we know, the effect of analytical performance goals for lipid assays on current guideline-recommended clinical management has not yet been evaluated.

Calculation of LDL-C is known to be particularly error-prone, especially when TG concentration is high or LDL-C is very low (8, 12). Alternatives to cLDL-C, such as direct LDL-C (dLDL-C), apolipoprotein B (apoB), and non-HDL-C (nHDL-C), include the error of only 1 or 2 measurement procedures and apoB and nHDL-C are not affected by elevated TG (9, 13, 14). Cardiovascular risk assessment may be improved, not only by reducing the allowable error of lipid panel assays but also by selecting tests or measurement procedures with less error.

In this study, we used error simulation to quantify the percentage of individuals that may be misclassified and mismanaged, in terms of cardiovascular risk, due to allowable error in the lipid panel assays. We then evaluated the clinical impact of reduction in this error within what should be achievable given the current state of the art (15). We also evaluated the extent to which replacement of cLDL-C with dLDL-C or nHDL-C improved outcomes and whether serial sampling to reduce the effect of BV is effective.

Materials and Methods

Lipid panel test results and clinical data were obtained from the National Health and Nutrition Examination Survey (NHANES) database for 28 722 individuals collected from 2005 to 2020. Measured TC, HDL-C, and TG were available, whereas cLDL-C was calculated using the Friedewald equation (16), in accordance with the current guideline recommendation (1). Individuals 40 to 75 years of age were included, while those with TG >400 mg/dL (4.5 mmol/L), diabetes mellitus, or on lipid lowering therapy were excluded, leaving a total of 8506 participants for study. Work under this study was not considered to be human subject research and was exempted from review by the Institutional Review Board at the National Institutes of Health.

Accepting the baseline values recorded in NHANES as "true," we classified individuals into 4 risk groups, A to D, based on the 2018 Multisociety guidelines (Fig. 1) (1). Individuals with cLDL-C < 70 mg/dL (<1.8 mmol/L) or with low or borderline

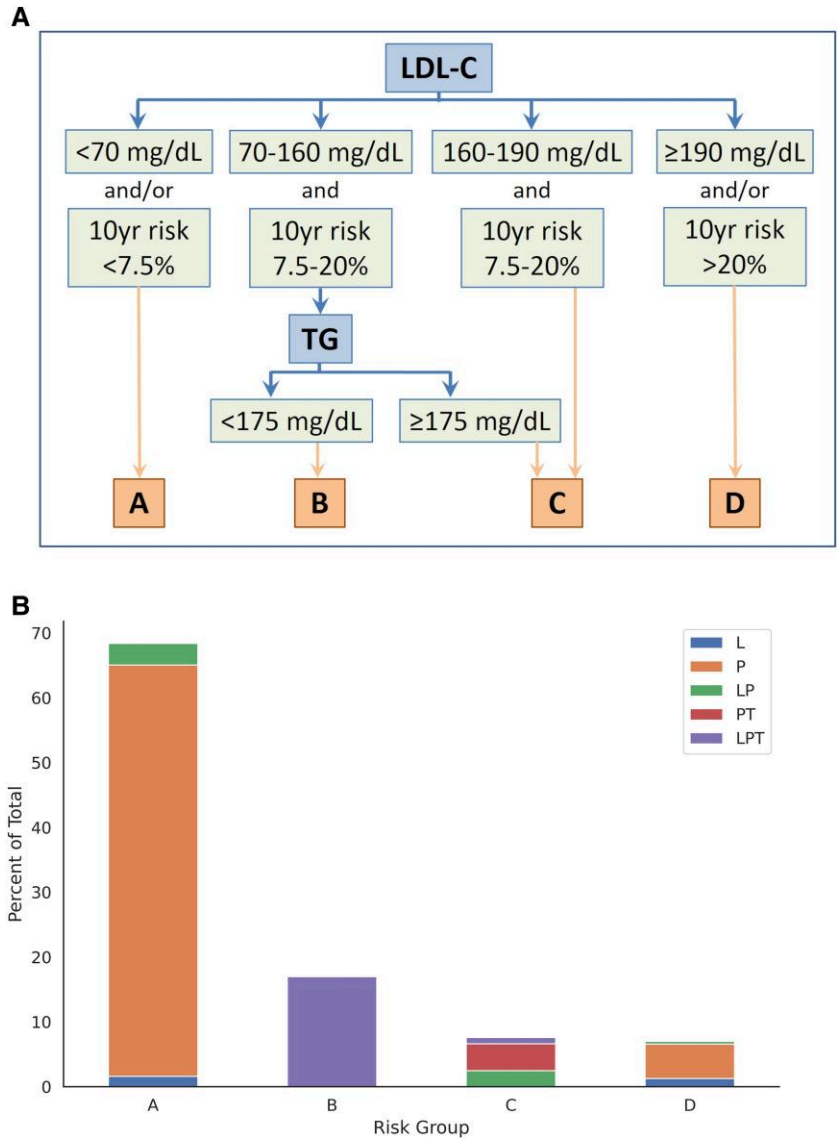

Fig. 1. (A), Lipid panel application in primary atherosclerotic cardiovascular disease prevention in nondiabetic adults, 40 to 75 years of age. To convert to SI units: Multiply cholesterol measures by 0.02586; multiply TG by 0.01129. Adapted from (1); (B), Classification of cohort into groups A to D based on original NHANES results. Abbreviations: L, LDL-C only; LDL-C, low-density lipoprotein cholesterol; LP, LDL-C and PCE; LPT, LDL-C, PCE, and TG; P, PCE only; PCE, pooled cohort equation; PT, PCE and TG; TG, triglycerides.

10-year risk scores (<7.5%) were categorized into group A. Individuals at intermediate risk (cLDL-C 70-190 mg/dL [1.8-4.9 mmol/L] and 10-year risk 7.5%–20%), were subdivided into groups B and C dependent on their cLDL-C and/or TG results. They were placed in group C if cLDL-C > 160 mg/dL (4.1 mmol/L) and/or TG >175 mg/dL (2.0 mmol/L), and in group B if not. These are the currently recommended cutoffs for the use of these tests as risk enhancers. Patients at the highest risk (cLDL-C > 190 mg/dL [>4.9 mmol/L] or 10-year risk > 20%) were categorized into group D. Secondary analyses were also performed on groups classified using nHDL-C decision limits in place of cLDL-C. The corresponding cutoff values for nHDL-C were 90, 190, and 200 mg/dL (2.3, 4.9, 5.2 mmol/L) (Supplemental Table 1).

To determine the current extent of potential misclassification, we applied the NCEP recommendations

for total allowable error in the lipid panel tests shown in Table 1 (5). Three hypothetical error scenarios were defined (Table 1). Each scenario was assessed with and without intra-individual BV, using values taken from the European Federation of Clinical and Laboratory Medicine Biological Variability database (11). As a baseline, we evaluated the effect of BV alone. In scenario 1, we excluded bias and applied analytical imprecision alone. In scenario 2, we maximized the positive error in cLDL-C and nHDL-C by applying positive proportional bias to the TC results, and negative proportional bias to the HDL-C and TG results, before applying BV and/or analytical imprecision. In scenario 3, we reversed this pattern to produce the maximal negative error in cLDL-C and nHDL-C. Proportional bias was applied using a linear equation. Biological variability and analytical imprecision were assumed to be normally distributed and were introduced sequentially using an inverse cumulative distribution function with "true" or biased values as the mean and the lower tail probability provided by pseudo-random number generation. Where the applied error caused TG to be >400 mg/dL (4.5 mmol/L), the Sampson equation was used to calculate LDL-C (17). This is in accordance with the US guideline's recommendation and thus simulates real-world practice (1). Any new values for TC, HDL-C, or TG <0 mg/dL (0 mmol/L) were corrected to 1 mg/dL (0.03 mmol/L cholesterol; 0.01 mmol/L TG). If cLDL-C was a negative value, this was considered to fall into the <70 mg/dL (1.8 mmol/L) group. We quantified the percentage of individuals that were misclassified due to the introduced error in each scenario, using both LDL-C and nHDL-C cutoffs, and repeated this 50 times to determine the mean and worst-case scenarios of the misclassification patterns. We analyzed the performance of dLDL-C assays in parallel with these scenarios, using cLDL-C as the "true" value and the error goals and BV for LDL-C provided in Table 1.

To select new performance goals, we examined the individual effects on misclassification of a range of analytical imprecision and proportional bias in each measurand, grouping first by cLDL-C and then by nHDL-C cutoffs. Based on these results, we selected new performance targets to propose for TC, HDL-C, and TG and reanalyzed our 3 scenarios using these (Table 1). Paired *t*-tests were performed between the pairs of current and proposed performance specifications applied to cLDL-C and nHDL-C-based classifications in each scenario and between paired cLDL-C and nHDL-C-based classifications using current and proposed performance specifications in each scenario.

We investigated the possibility of mitigating analytical imprecision and BV by simulating 10 analytical replicates and 10 sample replicates and using the means of the TC, HDL-C, TG, and dLDL-C results to determine the risk group based on LDL-C and nHDL-C cutoffs. All analyses were performed using Python 3.10 and the code may be freely obtained at https://github.com/JustineCole/PerformanceSpecs or https://doi.org/10.17605/OSF.IO/UX42S.

Results

Table 2 provides baseline characteristics of the cohort. Of the 8506 individuals included in the study, 4496 were female, 1924 were African American, 1932 were taking antihypertensive medication, and 1830 were smokers. Mean values for TC, TG, cLDL-C, and HDL-C were 203.3 mg/dL (5.3 mmol/L), 121.9 mg/dL (1.4 mmol/L), 122.7 mg/dL (3.2 mmol/L), and 56.1 mg/dL (1.4 mmol/L), respectively. Mean blood pressure was 125/74 mmHg and 25th and 75th percentiles for the 10-year ASCVD risk score were 1.6% and 9.6%, respectively, with a median of 4.3%.

Figure 1 shows how we modified the currently recommended algorithm for risk stratification to group individuals into the following 4 groups in order to simplify the analysis: (A) low risk, (B) intermediate-low risk, (C) intermediate-high risk, and (D) high risk. Based on the NHANES data, 68% of individuals fell into group A, 17% fell into group B, 8% fell into group C, and 7% fell into group D (Fig. 1). The results are color-coded in a stacked bar graph to indicate the proportion of individuals fulfilling each of the possible criteria to be placed in each risk category. For example, the vast majority of individuals in group A had a 10-year risk score <7.5% and very few had a cLDL-C < 70 mg/dL (1.8 mmol/L). Only a small minority shown in green fulfilled both criteria.

Figure 2A and Supplemental Fig. 1A show the average misclassification patterns, using cLDL-C-based classification, of 50 sample replicates due to BV alone and after introduction of the NCEP-recommended error maxima in the 3 scenarios described in the Methods section. BV alone resulted in misclassification of 6.3% of individuals on average. In scenario 1, we excluded any bias and examined the effect of analytical imprecision alone and with BV. Imprecision alone resulted in misclassification of 3.4% of individuals on average without BV and 7.1% with BV. Scenarios 2 and 3 maximize the positive and negative error in cLDL-C, respectively. Scenario 2 resulted in the highest misclassification rates, with more individuals being misclassified into higher risk groups, as would be expected. With no BV, 7.1% of individuals were misclassified on average, while 9.5% of individuals were misclassified when BV was included. On average, 31% of misclassifications in this scenario were into group D and approximately 16% of these individuals originated in group A. In the worst

Table 2. Cohort baseline characteristics.

	Mean	SD	Min	Max	25%	50%	75%
Age (years)	54.4	9.7	40	75	46	54	62
HDL-C (mg/dL)	56.1	17.1	6	226	44	53	65
TC (mg/dL)	203.3	39	79	463	177	202	227
TG (mg/dL)	121.9	65.7	18	400	75	105	151
LDL-C (mg/dL)	122.7	34.5	9	374.6	99.2	120.8	143.2
Non-HDL-C (mg/dL)	147.1	38.8	34	416	120	145	171
SBP	125.3	18.3	65	227	113	123	135
DBP	73.8	11.7	14	135	66	73	81
PCE	6.8	7.2	0.05	56.4	1.6	4.3	9.6
Total (n)	**Male (n)**	**African American (n)**	**On BP meds (n)**	**Smokers (n)**	**Diabetic (n)**	**On lipid meds (n)**	**TG >400 mg/dL (n)**
8506	4010	1924	1932	1830	0	0	0

To convert to SI units: multiply cholesterol measures by 0.02586; multiply TG by 0.01129.
Abbreviations: DSB, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PCE, pooled cohort equation; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

case of 50 replicates of this scenario, 10.1% of individuals were misclassified into a different group. In scenario 3, the average misclassification rate was 5.2% without BV and 7.6% with BV. In the latter scenario 42% of misclassifications were into group A and 5% of these individuals originated in group D. When nHDL-C is used in place of cLDL-C for classification, the highest average misclassification rates also occurred in scenario 2 but were lower, at 6.2% and 8.6% without and with BV, respectively (Supplemental Fig. 2A). In the worst case of this scenario, 9.2% of individuals were misclassified. BV alone accounted for an average misclassification rate of 5.7%. (Supplemental Fig. 1B).

Supplemental Fig. 3 shows the effect on misclassification of linear increases in analytical imprecision and bias in each assay. Imprecision in HDL-C and bias in TC had the greatest individual impacts on misclassification rate when using either cLDL-C or nHDL-C to categorize risk. When TG results were imprecise or inaccurate, nHDL-C-based classification was more reliable than cLDL-C. Note that TG is not used in nHDL-C calculation but does affect classification when used as a risk enhancer test as described in Fig. 1. We found no clear thresholds for any of the tests at which reductions in imprecision or bias resulted in a sharp decrease in misclassifications. Thus, we empirically selected achievable and impactful new error goals to present and propose (Table 1).

When we reduced the imprecision and bias as per the proposed error goals, there was up to 21% improvement in average misclassifications (Supplemental Table 2). By cLDL-C classification, the highest average misclassification rate was 8.6%, with a worst case of 9.2%, which is a 9% to 10% improvement (Fig. 2B). Similarly, the highest average misclassification rate using nHDL-C decreased to 7.7%, with a worst case of 8.3%, using our proposed performance recommendations (Supplemental Fig. 2B). These findings are summarized in Fig. 3, which provides the distributions of the original and improved misclassification rates across the 50 replicates with and without BV. In all scenarios, the misclassification rates noticeably improve with the proposed error goals, largely due to improved bias. Using nHDL-C resulted in lower misclassification rates in all scenarios compared to using cLDL-C. The results of paired *t*-testing were highly supportive these differences with all $P < 0.001$ (Supplemental Table 2). Figure 3 also shows that, using the current criteria, dLDL-C was superior to the calculated parameters in most cases. nHDL-C performed better than dLDL-C in all scenarios if the proposed specifications are met.

Finally, we investigated the possibility of reducing the impact of biological variability by simulating analytical replicates and sample replicates and assessing the effect of classifying based on mean results (Fig. 4). A single sample replication improved the cLDL-C misclassification rate from 7.1% to 5.4%. Three sample replicates reduced cLDL-C-based misclassification to a level similar to or lower level than 2 nHDL-C or dLDL-C sample

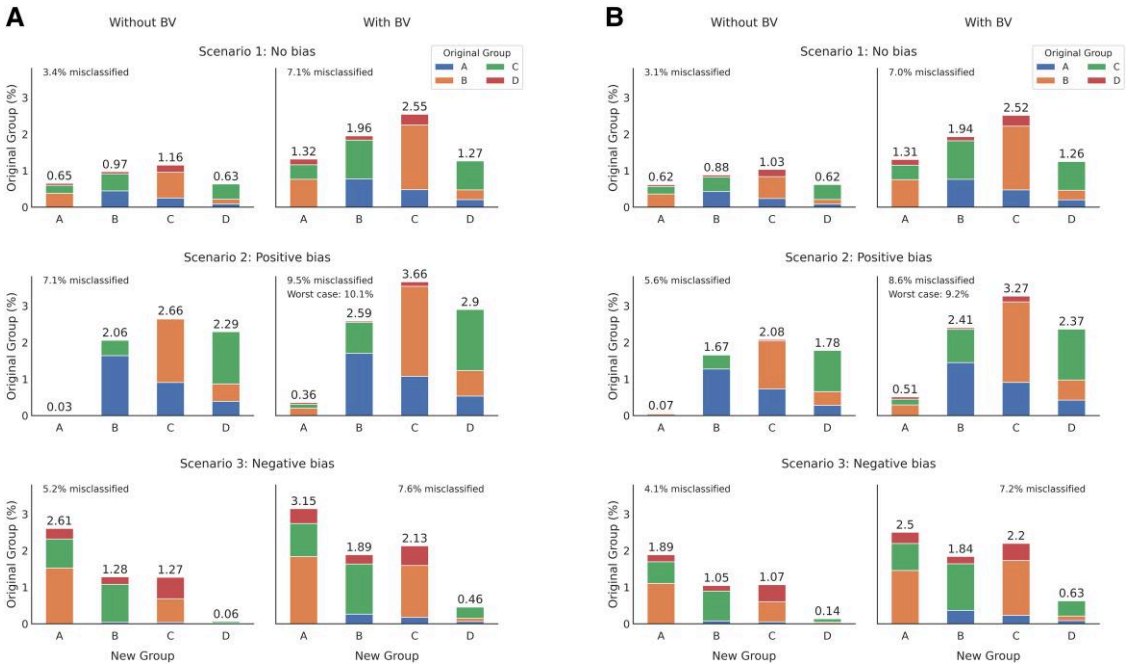

Fig. 2. Bar charts displaying the average and worst case of percent of individuals that were misclassified into each group using calculated low-density lipoprotein cholesterol-based risk classification in each scenario. Colors indicate the original risk group classifications before error was applied. (A), Misclassifications using current performance recommendations; (B), Misclassifications using proposed performance recommendations.

replicates. In contrast, analytical replication did not substantially reduce misclassifications.

Discussion

The limitations and challenges of standard lipid panel assays are well known among laboratorians (15), and it is widely understood that LDL-C, which is often calculated from these parameters, has inherent biological limitations as a marker of ASCVD risk and response to therapy (14). Nevertheless, the lipid panel parameters remain key biomarkers recommended in US (and other) guidelines for ASCVD risk stratification (1–3). Thus, any improvements that may be made to standardize their measurement and increase their reliability could be critical to patient care.

One major step toward standardization is improving the analytical performance required of assays (18). The Milan Hierarchy of 2015 specifies 3 models for designating analytical performance specifications. The highest model bases them on direct or indirect clinical outcomes. Performance specifications calculated from biological variability form the second model, while basing them on the state of the art is the least preferable model. If the state of the art prevents achievement of the clinically required quality, then improvement in technology is called for (10). The NCEP-recommended analytical performance criteria for lipid assays were based on expert opinion and were limited by the state of the art when they were set in 1990 and 1995 (4, 6, 7, 19). Biological variability-based performance specifications may be calculated (20) and tend to be more lenient than the NCEP-recommended specifications due to the wide BV of these assays (11). As far as we know, clinical outcomes-based analytical performance specifications for lipid panel assays have not previously been considered. In this study, we used accurate ASCVD risk classification, using the current guidelines, as an indirect clinical outcome to evaluate current and proposed performance specifications. This approach improves on previous evaluations of the impact of analytical errors in lipid assays (21) as the main outcome is a clinically meaningful parameter that directly affects patient management decisions.

Using the NCEP-recommended performance specifications and 3 hypothetical error scenarios of no bias, maximum positive bias on LDL-C, and maximum

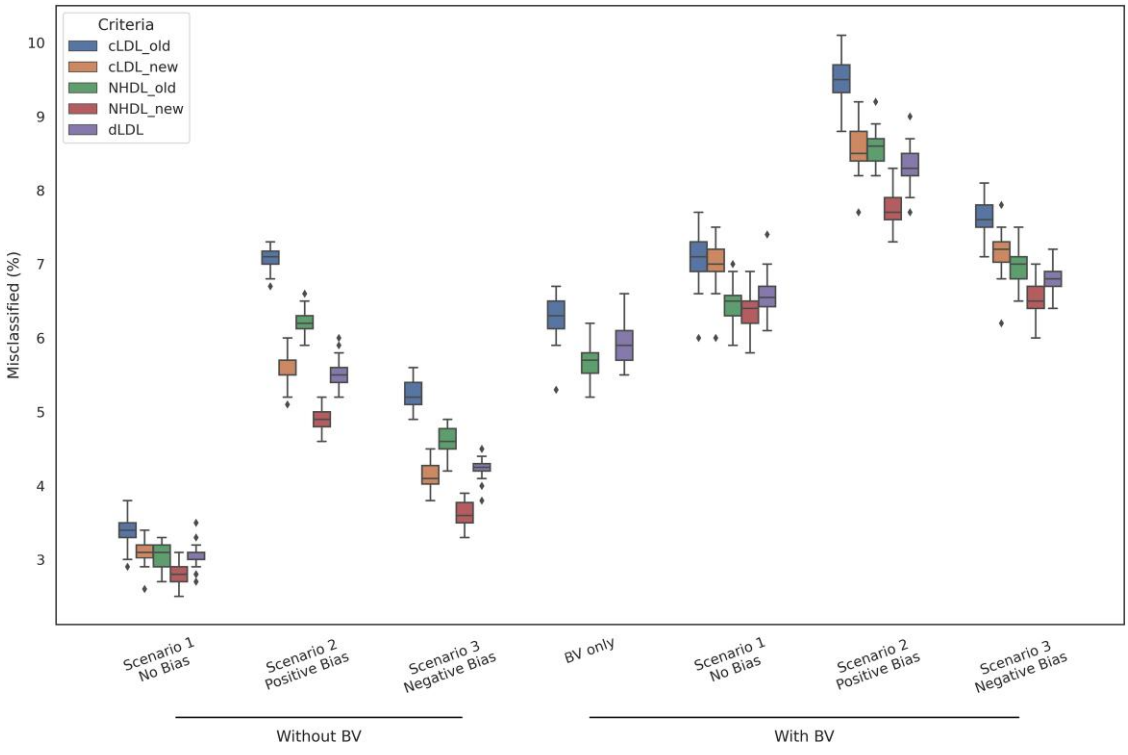

Fig. 3. Comparison of the distribution of misclassification rates across 50 replicates of lipid measurements affected by the current and proposed allowable error, in 3 error scenarios, with and without biological variability. Results were used to calculate LDL-C and non-HDL-C. Current performance criteria and biological variability were applied to direct LDL-C for comparison. Abbreviations: BV, biological variability; cLDL_new, calculated LDL-C, proposed criteria; cLDL_old, calculated LDL-C, current criteria; dLDL, direct LDL-C; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NHDL_new, non-HDL-C, proposed criteria; NHDL_old, non-HDL-C, current criteria.

negative bias on LDL-C (Table 1), we simulated the analytical error that could affect the lipid panel results of 8506 individuals from the NHANES database if the NCEP recommendations are met. These error-adjusted results were fed into the risk stratification algorithm presented in the current US guideline (1) to determine the extent to which the allowable error impacts the accuracy of risk classification, and thus management decisions, in ASCVD prevention. This process was repeated 50 times to find the average and worst case of the percentage of individuals that could be misclassified, and to what degree, in each of our hypothetical scenarios. When BV was included, the current allowable error led to misclassification rates of up to 10.1% when cLDL-C was positively biased. For those individuals who were grossly misclassified from the lowest to the highest risk group, this would have a major impact on their clinical management. With a negative bias on cLDL-C, in the worst case 8.1% of individuals were misclassified. Considering the number of lipid panel tests requested per year, a large number of individuals may be affected due to misclassification errors using the current goals for total analytical error.

By reducing the analytical imprecision to 3% across all assays and reducing the proportional bias by 1% in each assay, the misclassification rate using cLDL-C was reduced by up to 10%. Reduction in proportional bias appeared to have a greater impact than reduction in imprecision. In fact, simulated analytical replicates to reduce analytical variability did not reduce misclassification appreciably. The proposed goal for bias appears achievable at this time, based on the results of a recent College of American Pathologists survey (22). Analytical imprecision of 3% across assays also seems fair, given that analytical imprecision depends largely on the technical limitations of the instrument, assuming adequate quality control procedures are in place (23). We suggest that these proposed goals be the first steps in an ongoing improvement program to gradually lower allowable bias and reduce misclassifications, analogous

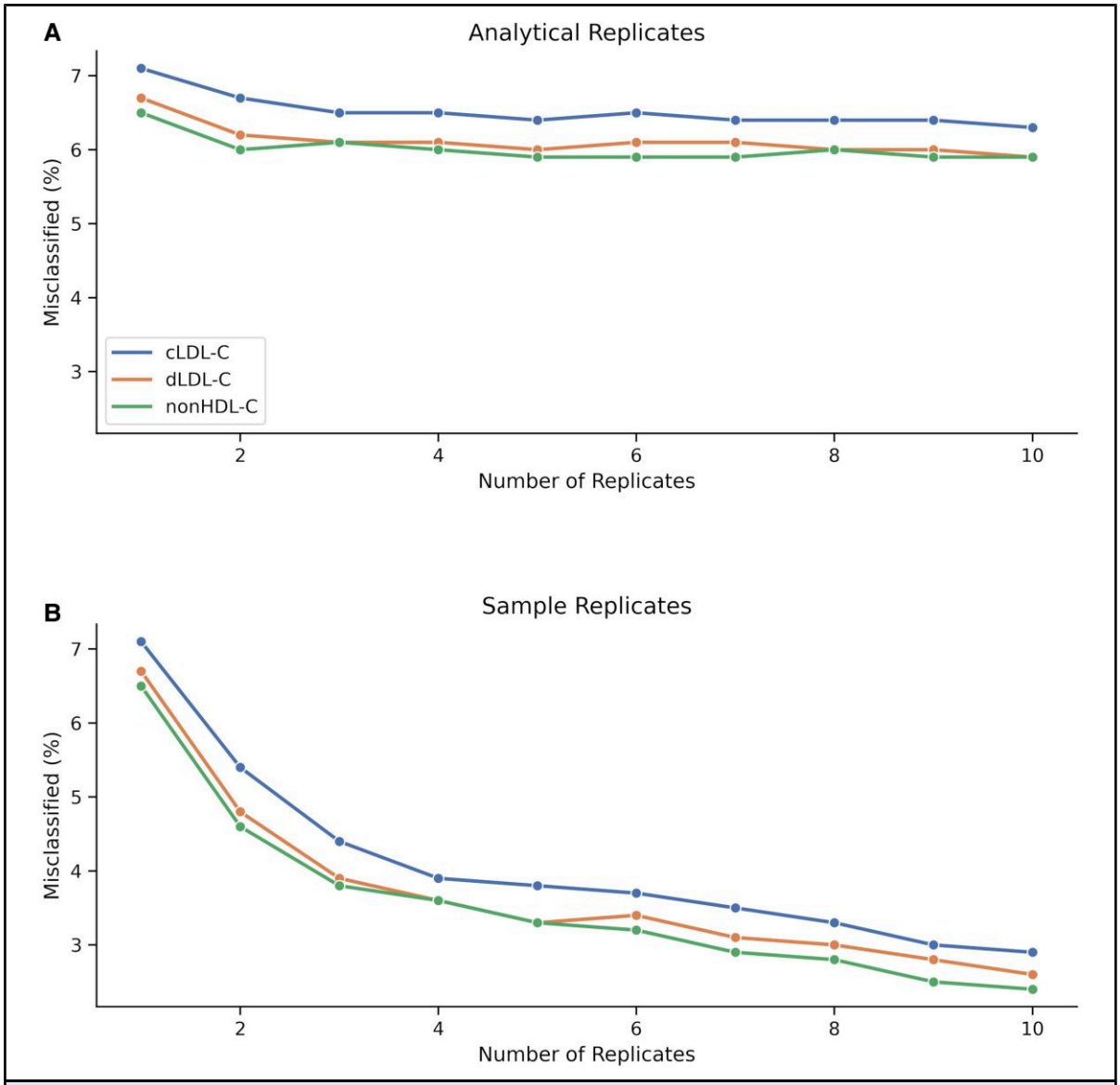

Fig. 4. Effect of simulated analytical and sample replicates to minimize variability of results. (A), Analytical replicates on single specimen per patient. Biological variability was applied once to baseline values. Imprecision added to this randomly for each replicate. No bias was applied; (B), Sample replicates to simulate collecting serial samples drawn on different days. Biological variability and imprecision were both applied randomly for each replicate. No bias was applied.

to what was achieved with hemoglobin A_{1c} in the diagnosis of diabetes (24). Any improvement might also extend to point of care lipid panel assays, which have poorer analytical performance compared to laboratory-based tests (25, 26). Without a continuing improvement program, there will be no incentive for diagnostic companies to refine their assays.

The high BV of plasma lipid concentrations is a major factor in the total error of lipid testing and subsequent misclassification. Calculated LDL-C is the most affected as it is based on 3 assays (TC, HDL-C, and TG) and carries the additive error of all of those measurements. To address BV, the US guidelines recommend that any decision to initiate a lipid-lowering drug should ideally be based on the mean result of at least 2 sample replicates for cLDL-C determination (1). We found that 3 serial samples, used to obtain mean values for cLDL-C calculation, lowered misclassifications by 2.7% and produced an

equivalent misclassification rate to 2 serial samples for dLDL-C or nHDL-C.

As an alternative to serial testing, biomarkers with less additive uncertainty could be used in place of cLDL-C. Although homogeneous assays have limitations that affect precision and accuracy (27), stratification based on dLDL-C resulted in a lower misclassification rate in our study using the current criteria, likely due to the fact that there is lower additive uncertainty. Similarly, using nHDL-C, which is dependent on only 2 assays, resulted in lower misclassification rates than did cLDL-C throughout all scenarios.

Non-HDL-C and apoB have several other advantages over LDL-C as a risk marker (14). Non-HDL-C includes very-low-density cholesterol and the cholesterol content of other atherogenic lipoproteins, thus providing a more holistic picture of risk (14). Its calculation depends on HDL-C measurement, however, which is the most error-prone of the lipid parameter measurements when using homogeneous assays, especially when TG are high (13, 15, 27). Based on recent reports, apoB may become the preferred parameter for risk stratification (28). It is based on a single measurement and is not affected by HDL-C assay error or the nonfasting state (13, 14). Importantly, it has a more direct biological relationship to ASCVD risk than do LDL-C or nHDL-C (14, 28). This is particularly relevant for patients with hypertriglyceridemia, which is associated with an increased number of small, cholesterol-depleted LDL particles and misleadingly low LDL-C concentrations (13, 29). ApoB also has the benefit of a lower BV (7.3%) compared with LDL-C (8.3%) and can be measured with better precision and less bias than cLDL-C (11, 29).

Limitations of our study include the fact that we did not have gold standard baseline results for TC, HDL-C, TG, and dLDL-C. The effects of the added error on the assumed baseline results are valid, however. Using the Sampson equation or Martin/Hopkins method to calculate LDL-C throughout our study may have improved the misclassification rate (17, 30), but, based on recent College of American Pathologists surveys, the majority of clinical labs are still using the Friedewald equation.

In summary, lipid panel results are applied to baseline risk assessments, as well as in follow-up visits to determine the need to commence statin treatment and to make decisions about increasing or reducing therapy. Given the prevalence of dyslipidemias, and the impact of lipid values on management decisions at the individual and societal levels, every incremental improvement made can have substantial positive impact. Based on our analyses, tightening the formal recommendations for analytical performance goals of lipids is advised. In the interim, individual clinical laboratories can adjust their internal performance goals. Laboratories may also elect to report nHDL-C values to encourage the use of this more analytically precise parameter until the clinical and laboratory communities are ready to adopt a better biomarker as the primary measurand for ASCVD risk assessment.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: apoB, apolipoprotein B; ASCVD, atherosclerotic cardiovascular risk; BV, biological variability; dLDL-C, direct low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NCEP, National Cholesterol Education Program; NHANES, National Health and Nutrition Examination Survey; nHDL-C, non-high-density lipoprotein cholesterol; PCE, pooled cohort equation; TC, total cholesterol; TG, triglyceride.

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Justine Cole (Conceptualization-Supporting, Formal analysis-Equal, Investigation-Equal, Validation-Lead, Visualization-Equal, Writing—original draft-Lead, Writing—review & editing-Equal), Maureen Sampson (Data curation-Lead, Formal analysis-Equal, Investigation -Equal, Visualization-Equal, Writing—review & editing-Supporting), Hendrik van Deventer (Investigation-Supporting, Writing—original draft-Supporting, Writing—review & editing-Supporting), and Alan Remaley (Conceptualization-Lead, Funding acquisition-Lead, Investigation-Equal, Methodology-Lead, Project administration-Lead, Supervision-Lead, Writing—review & editing-Equal)

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