Estimating and Minimizing Effects of Biologic Sources of Variation by Relative Range When Measuring the Mean of Serum Lipids and Lipoproteins

Gerald R. Cooper,1,3 S. Jay Smith,1 Gary L. Myers,1 Eric J. Sampson,1 and Erik Magid2

Biologic intraindividual variation (CVi) is a major source of inaccuracy in current lipid and lipoprotein measurements. Metaanalysis has been used to estimate the average CVi of serum total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc), and triglyceride (TG). These CVi values are larger than the National Cholesterol Education Program-accepted and -proposed analytic (CVa) goals. Measuring serial specimens reduces the error in determination of the mean concentration used in classification of the patient by cutoff points. We show (a) a convenient technique, based on the relative range, to qualitatively estimate and interpret biologic variation of TC, HDLc, LDLc, and TG, and (b) the number of serial specimens required to meet a total variation goal for measurements of mean lipid and lipoprotein values. A total variation goal has been selected that can be met by two serial specimens for a majority of individuals.

Indexing Terms: cholesterol/triglycerides/metaanalysis

Preanalytic sources of variation of serum lipids became of great concern to clinicians and laboratorians after these sources of variation were recognized as constituting >50% of the total variation that influenced the results of lipid measurements on serial specimens collected from one person (1). In a recent metaanalysis, we estimated the average intraindividual biologic variation (CVi) of serum total cholesterol (TC) to be 6.1%; for serum high-density lipoprotein cholesterol (HDLc), 7.4%; for serum low-density lipoprotein cholesterol (LDLc), 9.5%; and for triglyceride (TG), 22.6% (2). These CVi lipid values are larger than the National Cholesterol Education Program (NCEP)-accepted analytic (CVa) performance of 3% for TC (3) and the NCEP-recommended (and pending approval) CVa of 6% for HDLc, 4% for LDLc, and 5% for TG (4). These CVa criteria are based on experience by participating laboratories in the Centers for Disease Control and Prevention—National Heart, Lung, and Blood Institute Lipid Standardization Program (5) and consideration of the analytic criteria needed to classify a patient according to the recommendations of the Adult Panel II of NCEP (6). Because reducing intraindividual biologic sources of variation is possible only by changing lifestyle, minimizing total variation effects by measuring serial specimens is usually the most practical approach (7). The need for repeated measurements to assign risk accurately has been suggested in the NCEP Adult Treatment Panel II Guidelines (6) and has been emphasized by Bookstein et al. (8).

Improvements in methods, instruments, and calibrations have appreciably decreased the CVa of lipid measurements (9). It is timely to concentrate efforts on estimating the magnitude and effect of nonanalytic sources of variation on reported lipid results and to develop ways of reducing this within-subject variation (1). We have proposed a convenient technique, based on the relative range (RR), that will allow physicians to qualitatively estimate the CVi for serum TC and improve determination of the mean value of the individual (1). The RR evaluation requires the measurements of two or more specimens from a patient in a standardized lipid laboratory. Here, we extend the application of the RR model to the estimation and interpretation of biologic variation for HDLc, LDLc, and TG. Decreasing the variability of the determined mean value by use of multiple specimens also decreases the effect of biologic variation on the determination of the mean value. We show the number of serial specimens needed to reliably estimate with 95% confidence limits the mean lipid and lipoprotein value for one person.

Misclassification rates can be decreased and cost effectiveness improved if, when needed, additional serial samples of lipids are measured before treatment is prescribed (10).

Statistical Methods
Total Intraindividual Variation (CVi)

The total variation in the measurements of one or more specimens from one person is subject to both inherent biologic variation and analytic error. The biologic variation is estimated by analyzing multiple specimens from the same person. The analytic variation is usually estimated from repeated measurements of quality-control samples and of a single specimen. The best
estimate of an individual lipid value is the mean of two or more successive laboratory analyses. The CV_\text{T} of the mean lipid concentration is a function of the CV_b, the CV_a of the laboratory, the number of specimens (NS), and the number of replicate analytic measurements (NR) per specimen (see Appendix):

\[
CV_\text{T} = \frac{CV_a^2}{NR \times NS} + \frac{CV_b^2}{NS}
\] (1)

Using the above equation, we calculated for various lipids and lipoproteins the effect of multiple specimens and replicate analytic measurements on CV_\text{T} of mean lipids values for accepted analytic goals and average biologic variation by metaanalysis.

RR Determination for a Selected Mean and CV_\text{T}

We define RR as the difference between the lowest and highest concentration values observed for a person, divided by the mean of all the observed values. Expressing the difference between the values on a relative basis (percent difference) is useful, since the variability of specimen often increases with increasing concentration.

We determined the distribution and 95th percentile value of RR values for a selected mean and CV_\text{T} by performing a Monte Carlo simulation for a selected CV_a and CV_b. We generated 10,000 pairs of random observations from a gaussian distribution on the basis of a specified CV_b and CV_a combination for each analyte (corresponding to CV_\text{T}) and NS (two to five specimens from each subject). From these data, we calculated the distribution of the RR. The RR upper 95% tolerance limit was estimated as the 95th percentile of the RR distribution. This 95% tolerance limit is the cutoff point below which the subject's measured RR value would fall in 95% of the replicate analyses, given a specified CV_a and CV_b. If one assumes that the CV_a is correct, an observed RR value exceeding the 95th percentile tolerance limit implies higher biologic variability than expected.

Qualitative Assessment by RR Values

If the observed RR from a subject exceeds the RR 95% tolerance limit, then the person's biologic CV_b may be high, assuming standardized analytic measurements. To obtain a more precise estimate of the mean concentration, one must measure one or more additional specimens. Once an additional specimen has been measured, the RR should be recalculated to determine if it falls within expected variation limits.

Proposed Specifications for Analytic Error and Biologic Variation for Lipid and Lipoprotein Measurements

The analytic goals for CV_a in this study are the NCEP-approved goal for TC (3) and NCEP-recommended goals established by NCEP Lipoprotein Measurement Working Group, pending official approval by the NCEP Coordinating Committee (4). These analytic goals are considered attainable in well-controlled laboratories with existing methodology. The NCEP-recommended CV_a goals are 3% for TC (3), 6% for HDLC, 4% for LDLC, and 5% for TG (4).

A goal for minimizing effects of CV_b on CV_\text{T} of mean lipid values has not been officially accepted. At present, it seems reasonable to propose a goal that can be practically met for most individuals with two specimens; this is the number of specimens used by most clinicians and laboratorians. The biologic goal selected is to decrease the subject's CV_\text{T} of the mean of serial specimens to the population average CV_b (subject CV_\text{T} ≤ population mean CV_b). The current best estimate of the population mean CV_b is the value found by metaanalysis of previous publications (2). This goal is consistent with the recommendation of NCEP Adult Treatment Panels I and II, that the average of at least two measurements be used for clinical decisions.

Results

Variation Factors of CV_\text{T} on Mean Values of Serum Lipids and Lipoproteins

The effect of multiple specimens and analytic replicate measurements on CV_\text{T} of the determined mean value is shown in Table 1 for NCEP analytic goals (3, 4) and current biologic variation average values for the various lipids and lipoproteins (2). The CV_b values in Tables 1 and 2 are the mean values found by metaanalysis of the results of 30 published articles (2).

<table>
<thead>
<tr>
<th>Table 1. Effect of NS and NR on expected CV_a of mean values of serum TC, HDLC, LDLC, and TG for current analytic and biologic variability goals.</th>
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<tr>
<td>CV_a, %</td>
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<tr>
<td>n 1NR 2NR 1NR 2NR 1NR 2NR 1NR 2NR 1NR 2NR 1NR 2NR 1NR 2NR 1NR 2NR 1NR 2NR</td>
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<td>1 6.8 6.4 9.5 8.5 10.3 9.9 23.1 22.9</td>
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<td>4 3.4 3.2 4.8 4.3 5.2 5.0 11.6 11.4</td>
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<tr>
<td>5 3.0 2.9 4.3 3.9 4.6 4.4 10.4 10.2</td>
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</table>

CV_a are NCEP-accepted (3) and -proposed recommendations pending approval by NCEP Coordinating Committee (4); CV_b are average intraindividual biologic variability results of metaanalysis of 30 publications (2).

<table>
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<th>Table 2. Permissible RR upper-limit values for TC, HDLC, LDLC, and TG based on average CV_a from recent publications.</th>
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<td>RR</td>
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<tr>
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<tr>
<td>4 0.29 0.35 0.38 0.40</td>
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<tr>
<td>5 0.67 0.82 0.90 0.94</td>
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</table>

* Metanalytic average values of previous publication (2).

a This column's values are different from those of a previous publication (1) because the effect of NS on CV_a was only recently recognized.
Table 1 indicates that a large improvement in decreasing effect of CV_{T} on the mean value occurs from measuring additional serum specimens from the same person. Unless a large CV_{a} exists relative to the size of CV_{b}, additional replicate analyses of a specimen in a standardized laboratory are not appreciably more precise than the single analysis of a specimen. Since CV_{b} does not change unless lifestyle changes or subclinical disease occurs, a practical goal is to estimate the mean intraindividual value of serum lipid or lipoprotein with a CV_{T} equal to or less than the average CV_{b} (2). Thus, using the goals of CV_{T} ≤ (mean) CV_{b} of metaanalysis, two specimens are needed to gain 95% confidence in a mean lipid value of TC (CV_{T} ≤6.1%), HDLC (CV_{T} ≤7.4%), LDLC (CV_{T} ≤9.5%), and TG (CV_{T} ≤22.6%). Table 1 indicates that results of a single specimen cannot meet these criteria for the reported result. The use of two specimens actually lowers the CV_{T} to 4.7% for TC, 6.8% for HDLC, 7.3% for LDLC, and 16.0% for TG for single analyses of the specimens. A third specimen permits considerable further reduction of the CV_{T} for all lipids.

The interrelation of CV_{T}, CV_{a}, and number of specimens is illustrated in Fig. 1 for TC, HDLC, LDLC, and TG.

Fig. 1. CV_{T} of mean concentration as a function of CV_{a}, the number of specimens, and CV_{b}.
The range of abscissa values corresponds to that found in a recent metaanalysis. The vertical line on the abscissa indicates the mean CV_{b} found in the metaanalysis. The CV_{T} goal (horizontal line) corresponds to CV_{T} ≤ mean CV_{b}. At mean CV_{b}s and CV_{a}s, two specimens would be required to reduce the CV_{T}s to <CV_{b}s (goals) for TC (A), HDLC (B), LDLC (C), and TG (D).
ARR Upper-Limit Values for Average Metaanalytic Estimates of Biologic Variation and Standardized Analytic Procedures as Functions of NS and NR

The 95% upper limit of RR is presented in Table 2 for the metaanalytic average of biologic variation for lipids and lipoproteins when analyses are performed in a standardized laboratory, and for different numbers of serial specimens from the same person. For example, when two specimens are collected from the same person for serum TC analysis, the RR values for the metaanalytic mean CVb of 6.1% and analytic CVb of 3% will have a RR between 0 and 0.19. When the metaanalytic average CVb is used, the average upper limit RR for two serial specimens from the same person is <0.19 for TC, ≤0.27 for HDLC, ≤0.29 for LDLC, and ≤0.67 for TG. Persons with observed higher RR values will need more than two serial specimens to attain the desired CVT, for the reported mean result (i.e., measuring additional specimens lowers the CVT of the mean concentration estimate) as illustrated in Table 1 (1). The increase in RR with increasing number of specimens results from the expected increase in range with the larger number of samples. This is not related to a decrease in effects of biologic variation on the mean value by use of an increasing number of specimen analyses.

Discussion

The fact that the results of TC and other lipid measurements of some persons are highly variable is a real concern to clinicians and laboratorians. Analytic precision and accuracy in reported results have improved because of remarkably improved diagnostic products and instrumentation and because of stimulation from the NCEP recommendation that uniform cut-points be used when interpreting reported lipid results.

CVb is now recognized as a major contribution to CVT of (serially collected) specimen lipid results (1, 11–14) and varies considerably among individuals. Because of this, the effect of inherent biologic variation should be evaluated, and minimized if high, before the NCEP cut-points are used in classification of the patient. The effect of biologic variation may be minimized by standardizing the collection procedure and increasing the number of collected specimens. Biologic variation itself cannot be eliminated, however, and standardizing collection protocols may not yield a sufficiently low variation for some persons. To quickly estimate and consider what is needed to minimize the effect of biologic variation, we recommend that clinicians and laboratorians use a surrogate measure (RR) with two specimens. Provided this measure indicates acceptable biologic variation from two specimens, no additional specimens are required. If excessive variability is indicated by the RR, clinicians should collect additional specimens to measure the mean of the lipids within a proposed goal with 95% probability. Reliable estimates of the subject’s average lipid values thus may require measurements on repeat specimens.

We selected the RR as a surrogate indicator of the effect of the biologic variation on accuracy of mean value because it is convenient, easily understood, and has a known relation to SD (15). It also adjusts the total variation for the expected increase in difference of values as the mean concentration increases. The range of quality-control measurements has been used for ~40 years as a measure of analytic precision in clinical laboratory procedures (16). In control reference materials, range values reflect the concentration and the precision of the analytic diagnostic system. In serial specimens from the same person, the range of reported values is a function of effect of biologic concentration and variation as well as of analytic precision. RR therefore is applicable to estimation of effect of biologic variation on the mean value if the analytic error is known or is estimated for the laboratory analytic system.

The RR determined from a limited number of specimens does not provide a highly accurate assessment of the CVT. However, we believe that this is offset for the following reasons: First, the RR focuses attention on biologic variation and, at a minimum, requires two specimens, reducing sizeably the measurement error (SD) by 1/√2. Second, even in the case of lower-than-average biologic variation, samples can vary at random considerably; for example, one sample result could be statistically low (in the lower tail of the distribution) and the next statistically high (in the upper tail). The RR therefore has a good chance of detecting this situation and would require collecting an additional specimen that is likely to yield a more representative value.

The CVT, when mean values are involved, is related to CVb as shown in Eq. 1. Because CVb makes an inherent, relatively stable contribution to the variability of a person’s true lipid serum concentration, the contribution of CVb to the intrasubject lipid variation can be decreased only indirectly through factors that can affect the CVT. By increasing NS, the contribution of CVb to CVT is decreased and, with a sufficient number of specimens, the CVT can be decreased to meet the goal of CVT ≤ mean CVb. The equation for CVT also shows that the contribution of CVb can be decreased by increasing both NS and NR; however, increasing the latter is not as efficient as increasing NS. Table 1 illustrates the changes exerted on total CVT by decreasing the contribution of CVb through increasing NS from the same subject, a procedure that is more useful when CVb is large.

To determine if a patient’s biologic variation is excessive, a clinician or laboratorian can quickly calculate RR from the difference (range) of two specimens (or more), divide by average of the specimens, compare with the appropriate RR in Table 2, and if the calculated RR is less than the respective upper limit, be assured with 95% probability that the determined mean value lies within the CVT based on upper-limit analytic and biologic variation goals. The previously published results on TC (1) are slightly different from the first column of
Table 2 because CVₜ of 6.1% from metaanalysis is used instead of 6.5% previously, and additional simulations were done to improve accuracy.

Different levels of (mean) biologic variation may be postulated for experimentally controlled study types as well as for randomly selected individuals. For example, studies in which the subject's diet is carefully controlled probably yield lower (mean) CVₜ and corresponding CVₑ and RR values than studies in which diet is not controlled. To help evaluate this effect on the specimen requirements, one can show CVₑ (ordinate) as a function of the CVₑ range (Fig. 1A-D) and demonstrate the effect of the number of measured specimens for TC, HDLC, LDLC, and TG. The CVₑ for each figure is the NCEP tentative analytic goal (pending approval) (17) listed in Table 1; we assume one laboratory measurement per specimen.

The abscissa of each figure corresponds to the range of CVₑ found among studies in the metaanalysis (2); the vertical line is positioned at the mean CVₑ. It indicates the current best estimate of an average person's inherent biologic variation. Using Fig. 1A for TC as an example, we find that the mean CVₑ falls at 6.1% and that two specimens are required to maintain the total CVₑ below a target of 6.1%. For a person with previously known CVₑ at only 4%, we find that a single specimen could be adequate to achieve a 6.1% total CVₑ. If a person has greater biologic variation, say, 8% for TC, three specimens are required. In general, for most subjects, two specimens are adequate to meet the target CVₑ (when CVₑ ≤ mean CVₑ) values for TC, HDLC, LDLC, and TG. Using these figures, investigators can estimate the number of required specimens per subject for different types of studies. We regard these numbers as the minimal number of specimen replications required for 95% probability of CVₑ ≤ mean CVₑ. Other study requirements may indicate that a greater number of specimens is needed.

The NCEP-accepted and -proposed goals are not always consistent with the medical usefulness goal of CVₑ ≤ 1/2 CVₑ (18) and that of the NCEP Adult Treatment Panel, that the critical difference between two serial specimens falls within 30 mg/dL (0.78 mmol/L) for both TC and LDLC (6). Table 3 summarizes goals and total error limits, taking into account NCEP analytic goals and metaanalytic estimates of biologic variation.

The NCEP total error limits, for example, 8.9% for TC, reflect the analytic error associated with measuring a single specimen; they do not take into account biologic variation. Applying medical usefulness criteria (CVₑ ≤ ½ CVₑ) to the metaanalytic mean CVₑ, we find relatively good agreement with the NCEP CVₑ recommended values, except that the medical usefulness criteria yield a value about twice that of the NCEP for TG. Including expected mean biologic variation results in increases in the observed total error. The right-hand column of Table 3 gives the total expected percentage variation for the mean concentration expected within 95% confidence. This column reflects our proposed serum lipids goals stated as total error limits when analytic bias and precision and biologic variation are included. The third column of this table is highly important for proficiency testing and the last right-hand column is important for the clinician.

Using the total error limits reflecting all sources of variation from Table 3, we found for TC that the mean of two specimens from a person truly at 240 mg/dL (6.2 mmol/L) will fall within ± 30 mg/dL (0.78 mmol/L) of the true result with 95% confidence. We find a <1% chance of misclassifying the patient as <200 mg/dL (5.2 mmol/L). For a person truly at 200 mg/dL, the mean of two specimens has a <1% chance of falling at or >240 mg/dL. This indicates a <1% chance of misclassifying a patient into the high-risk category. These results indicate that using the NCEP-proposed analytic recommendations and minimizing the contribution of biologic variation to at least the effect of the mean CVₑ are practical criteria for reported results from lipid and lipoprotein measurements at the present time.

The RR is capable of detecting differences in results of two specimens outside the 95% probability of expected differences. The use of RR appears advantageous because it (a) avoids calculation of the CVₑ, (b) cancels out the effect of concentration on the standard deviation, (c) makes available a technique able to quickly estimate the effect of biologic variation from the use of only two to four specimens, and (d) reminds clinician and laboratorian of potential effects of biologic variation on reported lipid results.

Appendix
Explanation of Expression for CVₑ

Biologic variation is usually expressed as SD/X = CVₑ; therefore, CVₑ is proportional to the variance, (SD)². The variation of X for multiple measurements is (SD)²/n, or CVₑ/n. Assuming both analytic and biologic sources of er-

<table>
<thead>
<tr>
<th>NCEP-proposed precision</th>
<th>NCEP-proposed bias, %</th>
<th>NCEP-proposed total analytic error, % (95% limit: single specimen, no biologic variation)</th>
<th>Mean CVₑ, % (metaanalysis)</th>
<th>Medical usefulness allowable CVₑ, % (18) (based on metaanalysis CVₑ)</th>
<th>Total % error (NCEP total analytic error + biologic variation)</th>
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<tbody>
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<td>3</td>
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<td>5</td>
<td>15</td>
<td>22.6</td>
<td>11.3</td>
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</tbody>
</table>

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ror, we obtain the total variance for a single measurement:

\[ (SD)^2_{\text{TOTAL}} = (SD)^2_{\text{BIOLIC}} + (SD)^2_{\text{ANALYTIC}} \]

or

\[ CV^2_{\text{TOTAL}} = CV^2_b + CV^2_a \]

Using multiple specimens reduces the biologic component \((CV_b)^2\) by a factor of 1/NS.

The analytic component \(CV_a^2\) is reduced both by 1/NR and by 1/NS because there is variation of the mean reported result of at least two specimens and the NS reduction applies to the analysis of each specimen. The total analytic reduction becomes 1/NR \(\times\) 1/NS. \(CV_T\) represents the CV of the mean value of the number of specimens and replicates. Thus, the total variation becomes

\[ CV^2_T = \frac{CV^2_a}{NR \times NS} + \frac{CV^2_b}{NS} \]

References