Fundamental Problem with Assays of Apolipoprotein A-I

To the Editor:

I would like to comment on three papers on assays of apolipoprotein (apo) A-I, published in Clinical Chemistry, by Albers and Marcovina (1), Bachorik and Cloey (2), and Wang et al. (3). Marcovina and Albers (1), reporting on the conclusions of the International Federation of Clinical Chemistry meeting in Vienna on standardization of apolipoprotein assays, indicated that the major discussions revolved around preparation of primary standards, reference materials, and reference ranges, with less discussion as to exactly what was being measured. Bachorik and Cloey (2) compared the Beckman and Behring immunonephelometric (INA) kits and the Tago radial immunodiffusion (RID) kit methods for assaying apolipoproteins. They found correlation coefficients between the methods for apo A-I of about 0.7, a degree of correlation that the authors concluded was only marginally adequate. Actually, because the degree to which variation in y is dependent on x is given by the square of the correlation coefficient, a better conclusion would be that the relationship between the methods was inadequate, with between-method agreement only about 50% of the time.

These results reflect a fundamental and widespread problem in measuring apo A-I. Lipoproteins are composed of lipids and proteins, with an inner core of the less-polar lipids, and an outer region containing the more-polar lipids and apolipoproteins in a configuration wherein the nonpolar portions of the proteins extend towards the core. When polyclonal antibodies are being produced for assays, T-cells digest the injected antigens so that antibodies are produced against many polypeptides of apo A-I, those that are hidden as well as those that are exposed in endogenous HDL. Unless the apolipoprotein antigens in endogenous HDL can be sufficiently exposed during an assay to react completely with the antibodies, the amount of reaction will be biased by the peculiar conformation of lipid and protein. Given this situation, it is unlikely that effective immunoassays of apo A-I can be developed.

Mao et al. (4) demonstrated that 15% of apo A-I in untreated endogenous HDL is immunoreactive with polyclonal antisera, the rest being hidden; they noted the poor parallelism between assays of apo A-I in plasma and assays of purified apo A-I unless the plasma is pretreated with a denaturing agent that thoroughly exposes the antigenic sites. Although in a previous publication (5) we were able to demonstrate a much higher correlation (0.92) between results by the Beckman INA and the Tago RID methods, we found poor parallelism between apo A-I in plasma and purified apo A-I when the Beckman assay was conducted with pretreatment with the manufacturer's buffer, which contained Triton X-100. However, we did obtain good parallelism when we pretreated the plasma with Tween 20, which sufficiently exposes apo A-I for accurate immunological assay (6). As Albers and Marcovina indicated (1), good parallelism with a primary purified apo A-I standard is a necessary (but not sufficient) criterion for assessing whether or not an assay is completely measuring the apolipoprotein in endogenous HDL.

As in many other papers comparing apolipoprotein assays, Bachorik and Cloey (2) did not examine the question of parallelism with pure apo A-I, which is the mainstay for comparing immunological compatibility. Many authors comparing apolipoprotein assays have used plasma pools obtained from the Centers for Disease Control or other sources, standards that may not be adequately prepared themselves or assayed in a proper matrix environment.

The paper by Wang et al. (3) appears to complicate the problem even more. They suggest that the immunoreactivity of apo A-I varies with the degree of oxidation of the molecule and that, depending on the oxidative state of apo A-I, Tween 20 is apparently less effective than other detergents at unmasking antigenicity. Unfortunately, the data were not conclusive, because they did not examine the effect of oxidation on purified apo A-I.

It seems premature to be comparing and assessing methods when we are not yet certain which methods can measure apo A-I with any degree of certainty. The great discrepancies obtained in clinical trials—with some trials showing great discrimination (7), and other trials poor discrimination (8), between the apo A-I concentrations of persons with and without coronary disease—may quite possibly be due to the various degrees of antigen exposure.

Evidence indicates that antibodies directed against only surface antigens effectively measure the concentration of apo A-I in HDL particles (4). Assuming that every apo A-I molecule has one common antigen exposed, perhaps monoclonal surface-specific antibodies can provide an adequate method for measuring apo A-I for widespread use. A great amount of additional research seems necessary to resolve this problem.

References
8. Aro A, Soimakallio S, Voutilainen E, et al. Serum lipoprotein lipid and apoprotein

Stanley S. Levinson
Dept. of Pathol., Univ. of Louisville, and
The Laboratory Service
800 Eorn Ave.
Veterans Administration Med. Center
Louisville, KY 40206-1499

Apolipoprotein A-I Concentrations in Serum: A Pragmatic Approach to Assessing Risk of Heart Disease

To the Editor:

Several studies have shown that apolipoproteins B (apo B) and A-I (apo A-I) are significant predictors of coronary heart disease (CHD) (1). Unfortunately, problems related to the standardization of the assay procedure for apo B and Apo A-I hinder a correct and widespread clinical use of these tests.

At present, the reference intervals for apo A-I are method dependent, so that, unlike the case with high-density lipoproteins (HDL-C), no cutoff limit for CHD risk has been selected. Because HDL values below the 10th percentile are considered a risk factor for CHD (3) and because apo A-I concentrations are strictly related to those of HDL-C (4), recently Albers and Marcovina (5) suggested that "apo A-I concentrations below the 10th percentile are a risk factor for coronary heart disease."

When a single cutoff value is used, as in the case with HDL-C, the reference limit generally is drawn from an epidemiological retrospective analysis or from cases selected clinically (patients with or without CHD). However, we suggest here a different approach to the problem. We measured HDL-C as described previously (6) and apo A-I with a Behring nephelometer, according to the manufacturer's instructions and using polyclonal antisera. We used these values to prepare a linear-regression curve for 286 serial assays of apo A-I (g/L) and HDL-C (mmol/L) in sera selected without conscious bias (Figure 1). The slope was 0.521 and the intercept 0.78 (r = 0.887).

Line A in Figure 1 is drawn at the HDL-C value 0.90 mmol/L, which is considered the cutoff limit for CHD risk (3). Line B is drawn just below the lowest apo A-I value measured in samples with HDL-C = 0.90 mmol/L. Thus we consider this value for apo A-I, 1.10 g/L, the reference limit for CHD risk in this population.

Furthermore in a retrospective analysis of 1023 paired values for apo A-I and HDL-C, we never found apo A-I concentrations <1.10 g/L associated with HDL-C values >0.90 mmol/L. Apo A-I values exceeding the cutoff limit are not excluded in samples with HDL values <0.90 mmol/L; in such cases, the two measurements can be considered independent factors of risk.

We do not suggest that this particular value of apo A-I be used as a reference limit generally. Instead, because apo A-I values are method dependent, this cutpoint should be established in each laboratory, according to results for that laboratory's own samples, and for an adequate number of samples, at least 200 paired values.

This approach is feasible in all laboratories and avoids the more difficult, time-consuming, and expensive identification of population-based reference intervals.

References

Ugo Lippi
Donatella Bertolini
Maria Stella Graziani
Ospedale Civile Maggiore
Piazzale Stefani, 1
37126 Verona, Italy

Graphic Representation of Reference Intervals

To the Editor:

According to the IFCC-approved recommendations (1), the central 95% reference limits of laboratory tests should be reported with their 90% confidence intervals to facilitate comparison of data from different studies. For reference values having a gaussian distribution, the confidence intervals would be identical and situated symmetrical above and below the reference limits. For a nongaussian distribution and nonparametric estimation of reference limits, the confidence intervals, taken from a table (2), would be neither identical nor symmetrical. Their diversity would depend mainly on the shape of distribution (right or left skewness), sample size, and analytical imprecision.

In routine medical practice, however, it is difficult for all these statistical parameters to be appropriately used. The reference interval is usually perceived as homogeneous, thus obscuring meaningful information as to how unusual a patient's result could be. A graphic representation (along with numerical values) would better adapt specific reference intervals to the requirements of the natural stages of human perception: filtering, simplification, coding, and grouping, to help recognition and segregation of laboratory data (3).

I propose here a graphic model for the reference interval, simple and informative enough to help in interpreting the reference values and the individual results falling between or near the reference limits. The model (Figure 1) presents the reference interval (central 95 percentile fraction of reference values) as a long vertical line.