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Lipoprotein(a) Level as a Predictor of Cardiovascular Disease and Small Apolipoprotein(a) Isoforms in Dialysis Patients: Assay-Related Differences are Important

J. Craig Longenecker^{1,2}, Josef Coresh¹, Michael J. Klag¹, Neil R. Powe¹, Nancy E. Fink¹, and Santica M. Marcovina³

¹Johns Hopkins University, Baltimore, MD.

³Northwest Lipid Research Laboratories, University of Washington, Seattle, WA.

Abstract

Background—Lipoprotein(a) assays sensitive to apolipoprotein(a) size may underestimate associations of lipoprotein(a) with cardiovascular disease (CVD) and low molecular weight (LMW) apolipoprotein(a) isoforms. This study among 629 dialysis patients compares the value of two lipoprotein(a) assays in predicting CVD events and small isoforms.

Methods—Lipoprotein(a) level was measured by an apolipoprotein(a) size-insensitive ELISA and apolipoprotein(a) size-sensitive immunoturbidometric (IT) assay; and apolipoprotein(a) size by Western blot. Positive/negative predictive values (PPV/NPV) for small isoforms were calculated, and CVD events ascertained prospectively.

Results—The ELISA assay predicted CVD more strongly [Relative Hazard, RH=1.8; p=0.045, at the 85th Lipoprotein(a) percentile] than the IT assay (RH=1.3; p=0.37). The PPV for LMW isoforms using the ELISA (Whites, 98%; Blacks, 90%) were much higher than the IT assay (Whites, 75%; Blacks, 68%). Relative to the ELISA assay values, a positive bias in the IT assay values was seen for participants with larger apolipoprotein(a) isoforms, which may explain these findings.

Conclusions—When measured by an apolipoprotein(a) size-insensitive ELISA assay, but not a size-sensitive IT assay, high lipoprotein(a) levels predict both incident CVD and LMW isoforms in dialysis patients. Clinicians ordering lipoprotein(a) levels and research studies of lipoprotein(a) should determine if an apolipoprotein(a)-size related bias is present in the assay they use.

Keywords

lipoprotein(a); apolipoprotein(a) isoforms size; cardiovascular disease; dialysis; sensitivity; specificity

Corresponding Author: J. Craig Longenecker, MD, PhD, MPH, Faculty of Medicine, Department of Community Medicine, Kuwait University, P.O. Box 24923, Safat, 13110, Kuwait, Tel: 011-965-531-2000 x6534, Fax: 011-965-533-8948, E-mail: jlongene@hsc.edu.kw.

²Current Affiliation: Kuwait University, Kuwait City, Kuwait

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Disclaimers: None

Supplemental Material for Online Appendix

We are requesting that a Figure be included in an online appendix. It provides important data that are not able to be accommodated in the paper itself.

INTRODUCTION

The clinical usefulness of lipoprotein(a) measurement in dialysis patients has been questioned by some studies reporting that low molecular weight (LMW) apolipoprotein(a) isoform size, but not lipoprotein(a) level, predict coronary heart disease (CHD) [1,2] or total mortality [3]. Others report that although lipoprotein(a) level predicts cardiovascular disease (CVD) events, LMW apolipoprotein(a) size does so more strongly [4].

Interpreting lipoprotein(a) levels, however, is problematic owing to the proliferation of different lipoprotein(a) assays, particularly assays measuring lipoprotein(a) mass instead of concentration [5]. The lipoprotein(a) assays that measure mass utilize antibodies directed to epitopes present on Kringle-IV (K-IV) type 2 motifs, the number of which is genetically determined and varies widely between individuals. As illustrated in Figure 1, apolipoprotein (a) size heterogeneity may result in an underestimation of lipoprotein(a) level among individuals with smaller apolipoprotein(a) isoforms, and over-estimation with larger isoforms [6,7]. Even with the same lipoprotein(a) concentration, a sample with a LMW isoform will have fewer epitopes interacting with antibodies, thus generating a lower signal, and *vice versa*. Assays have been developed that avoid this bias by using a monoclonal antibody to a stable, non-repeating epitope present on the K-IV type 9 motif, thus accurately measuring lipoprotein(a) protein concentration and expressing the results on a molar basis [7,8].

This potential apolipoprotein(a)-size-related bias, if present, would bias the association between lipoprotein(a) mass and CVD events towards the null, assuming that some of the risk of CVD associated with lipoprotein(a) is mediated through LMW isoforms. However, no studies in the dialysis population have reported a head-to-head comparison of the two types of assays.

Furthermore, while it may be clinically useful to identify dialysis patients who have LMW apolipoprotein(a) isoforms, the method for determining apolipoprotein(a) size is technically difficult and not commercially available for clinical use. Because lipoprotein(a) levels are significantly higher in individuals with LMW apolipoprotein(a) isoforms sizes (compared to those with HMW isoforms), even in dialysis patients [9,10], lipoprotein(a) levels above a given cut-off may be able to identify LMW isoforms. To our knowledge, however, the predictive value of lipoprotein(a) level in identifying LMW isoforms has not previously been reported.

The first objective of this study of a large biracial dialysis cohort is to identify lipoprotein(a) cut-offs above which higher-risk individuals with both high lipoprotein(a) level and LMW isoforms can accurately be identified, and the associated diagnostic operating characteristics of an apolipoprotein(a) size-insensitive ELISA compared to an apolipoprotein(a) size-sensitive immunoturbidometric (IT) assay. The second objective is to compare the two assays in their ability to predict prospective CVD events. The *a priori* hypothesis was that, compared with the IT assay, the apolipoprotein(a) size-independent ELISA would more strongly predict both prospective CVD events and LMW isoforms.

METHODS

Study Design and Research Population

This study incorporates a cross-sectional and a prospective analysis of the Choices for Healthy Outcomes in Caring for ESRD (CHOICE), a cohort study of dialysis outcomes described previously [3,4]. Eligibility criteria included initiation of outpatient dialysis in the preceding three months, ability to give informed consent, age over 17 years, and ability to speak English or Spanish. Study methods adhered to the Declaration of Helsinki. The Johns Hopkins

University School of Medicine Institutional Review Board and the review boards for the clinical centers approved the study protocol, and written informed consent was obtained from all participants prior to enrollment.

From October, 1995, to June, 1998, 1041 incident dialysis patients from 19 states were enrolled at 81 dialysis clinics associated with Dialysis Clinic, Incorporated (DCI-Nashville, TN; n=923) and two other centers (n=118). A specimen bank was established among 95% (n=873) of the DCI participants, among whom apolipoprotein(a) isoforms size (n=871), lipoprotein(a) concentration (by ELISA; n=871) and lipoprotein(a) mass (by IT assay; n=848) were measured. This study, which directly compares the two assays, excluded 242 participants whose ELISA and IT assays were performed on samples from different blood draws (n=219) or did not have an IT assay performed (n=23), providing 629 participants eligible for analysis (422 White; 174 Black; 33 other).

Data Collection

Non-fasting, pre-dialysis, serum specimens were centrifuged within 30–45 minutes of blood draw and sent overnight on ice to the DCI Central Laboratory. Each blood draw was aliquoted into multiple vials and stored immediately at -80°C . Median time from first dialysis to blood draw was 4.4 months [IQR: 3.7–5.2 months].

Lipoprotein(a) mass was measured using the SPQ Test System (INCSTAR; Stillwater, MN) employing an immunoturbidimetric (IT) assay (units, mg/dl). Polyclonal antibodies recognize epitopes present on the repeating K-IV type 2 sub-units of apolipoprotein(a). Turbidity of antigen-antibody complexes is measured by light absorbance, and is compared to the reference standard. The C.V. ranges from 2.6% for high lipoprotein(a) levels (mean, 73.7 mg/dl) to 9.3% for low levels (mean, 6.4 mg/dl). Never-previously-thawed aliquots were analyzed in batches using the IT assay at the DCI Laboratory (median time from storage to analysis, 1.5 months).

Lipoprotein(a) protein concentration (nmol/L) was measured by direct binding double monoclonal antibody-based ELISA [7]. The detection antibody (a-40) is directed to a unique epitope present in apolipoprotein(a) K-IV type 9, making this immunoassay insensitive to apolipoprotein(a) size heterogeneity. The Coefficient of Variation (C.V.), based on five duplicate samples of varying lipoprotein(a) concentrations (12 to 120 nmol/L) in each ELISA plate, ranges from 4.0% to 6.7%.

Apolipoprotein(a) isoforms were characterized using a high-resolution SDS-agarose gel electrophoresis method followed by immunoblotting [11]. A consistent relationship between the number of K-IV motifs as determined by pulsed-field gel electrophoresis [12], and the relative migration of apolipoprotein(a) in agarose gel has been determined [11]. Therefore, we used a size designation based on the respective number of K-IV motifs. The C.V. for the apolipoprotein(a) isoform size in the CHOICE cohort was 11.7%. An exact match of the smallest allele size for the 49 blindly split samples was present for 48.9%, and a match ± 1 repeat was present for 93.8%. By convention, LMW apolipoprotein(a) isoforms size was defined as ≤ 22 K-IV repeats; and HMW isoforms as > 22 K-IV repeats.

For the ELISA and apolipoprotein(a) size determination, never-previously-thawed frozen aliquots were sent on ice to the Northwest Lipid Laboratory for analysis. Median time from storage to analysis was 21 months. Performance of the ELISA was blinded to the results of the apolipoprotein(a) size determination. Only aliquots for which there was a corresponding IT assay result from the same blood draw were used for analysis.

Clinical data were measured at baseline by chart review, as described previously [13]. For the prospective analysis of CVD events, patients without CVD at baseline were followed until fatal

or non-fatal myocardial infarction, cerebrovascular accident, coronary artery bypass, carotid endarterectomy, or amputation. Fatal and non-fatal outcome events were ascertained using a combination of Health Care Financing Administration hospitalization discharge data, hospitalization medical record adjudication, and dialysis record review, as previously described [4].

Statistical Analysis

Statistical analyses were performed with Stata© 8.0. *A priori* cutoffs for lipoprotein(a) level were the 15th, 25th, 75th, and 85th percentiles. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) at each cutoff were calculated using the dichotomized apolipoprotein(a) size variable (LMW/HMW) as the gold standard, and 95% confidence intervals using the binomial exact method.

Post-test probabilities were calculated using diagnostic likelihood ratios for each level defined by the four *a priori* cutoffs. Race-specific prevalence of LMW isoforms was used as the pre-test probability for LMW isoforms (Whites=0.41; Blacks=0.46). Pre-test odds were multiplied by the likelihood ratio to produce post-test odds.

To compare lipoprotein(a) mass to concentration, the ratio of the IT assay lipoprotein(a) mass value divided by the ELISA lipoprotein(a) concentration was used. Non-parametric methods were used to test for trend across apolipoprotein(a) size groups. Using LMW isoforms status as the binary outcome, Receiver-Operator Characteristic (ROC) curves for the two assays were constructed.

Prospective CVD analyses used Cox Proportional Hazards models. Survival time was defined as time from blood draw to outcome event or censoring. Covariates in the models included age, race, sex, baseline dialysis modality, comorbidity score, cause of renal disease, baseline CVD, total cholesterol, HDL-cholesterol, smoking status, systolic blood pressure, albumin, body mass index, creatinine, and C-reactive protein.

RESULTS

Demographic characteristics were similar to the U.S. dialysis population, as reported by the United States Renal Data System (USRDS [14]); with respect to median age (60 vs. 61 years, respectively), sex (54% vs. 53% male), race (28% vs. 29% Black), and cause of renal disease (diabetes, 46% vs. 42%; hypertension, 18% vs. 25%; glomerulonephritis, 15% vs. 9%; and other, 21% vs. 23%). The proportion of peritoneal dialysis patients (19%) is higher than USRDS (13%) because CHOICE over-sampled peritoneal dialysis patients. The median lipoprotein(a) concentration (ELISA assay) was much higher for Blacks (86 nmol/L), compared with Whites (36 nmol/L; $p<0.0005$); as was lipoprotein(a) mass (IT assay; 49 mg/dl vs. 24 mg/dl, respectively; $p<0.0005$). The prevalence of LMW isoforms among Whites was 41% and 46% among Blacks. Scatterplots of lipoprotein(a) level versus isoform size, stratified by race, are available in an online Appendix Figure.

Using the 85th percentile cutoff for the ELISA (Whites/Hispanics: 158.8 nmol/L; Blacks: 169.4 nmol/L), we calculated the overall specificity (358/362=98.9% [95% C.I., 97.2%–99.7%]), sensitivity (88/267=33.0% [27.3%–39.0%]), PPV (88/92=95.7% [89.2%–98.8%]); and NPV (358/537=66.7% [62.5%–70.6%]). In contrast, using the 85th percentile cutoff for the IT assay (Whites/Hispanics: 71.0 mg/dl; Blacks: 129.9 mg/dl), the specificity (336/362=92.8% [89.7%–95.3%]), sensitivity (69/267=25.8% [20.7%–31.5%]), PPV (69/95=72.6% [62.5%–81.3%]); and NPV (336/534=62.9% [58.7%–67.0%]) were all lower than for the ELISA.

Table 1 presents the operating characteristics for lipoprotein(a) level at the 15th, 25th, 75th, and 85th percentile lipoprotein(a) cutoffs for the two assays, stratified by race. At the 85th percentile cut-offs for Whites, the specificity and PPV of the ELISA in identifying LMW isoforms were 99.6% and 98%, respectively; whereas the corresponding figures for the IT assay were lower at 94% and 75%, respectively. The comparison of the two assays among Blacks was similar to Whites, but with weaker specificity and PPV.

Sensitivity and NPV were also higher for the ELISA than for the IT assay. However, sensitivity and NPV at levels below the 15th percentile were stronger among Blacks (95% and 84% using the ELISA, respectively) than among Whites (90% and 71%, respectively).

Similarly, Table 2 shows that the ELISA is more accurate than the IT assay in identifying LMW isoforms. The diagnostic likelihood ratios are much higher for the ELISA than the IT assay. The post-test probability is 0.95 among Whites with concentration > 158.8 nmol/L (the 85th percentile for Whites), and 0.93 among Blacks with levels > 169.4 nmol/L (the 85th percentile for Blacks). Both are much higher than the corresponding post-test probabilities using the IT assay. When using the ELISA, the post-test probability of LMW isoforms for levels below the 15th percentile was 0.29 for Whites and 0.11 for Blacks,.

The ROC curves for the two assays are presented in Figure 2. The area under the curve for the ELISA test is 0.74 [95% C.I., 0.70–0.78], compared to 0.66 [0.62–0.71; $p < 0.00005$] for the IT assay. Importantly, the ROC curves are skewed, and most of the improvement of the ELISA over the IT assay occurs in the area of the highest specificity (the left edge of the curve), which most affects the ability to “rule in” LMW isoforms (PPV).

Table 3 presents the median lipoprotein(a) values across the four quartiles of apolipoprotein (a) isoform size for both assays. The ratio for the ELISA is approximately twice as large as the ratio for the IT assay in both Blacks and Whites, reflecting a much stronger association between lipoprotein(a) level and apolipoprotein(a) size for the ELISA compared to the IT assay.

Figure 3 presents box plots of the IT:ELISA ratio [ratio of lipoprotein(a) mass to concentration], stratified by race and apolipoprotein(a) size categories. This ratio increases with each larger apolipoprotein(a) size category, ranging in Whites from a median ratio of 0.33 in the smallest apolipoprotein(a) category to a median ratio of 0.74 in the largest ($p < 0.0001$; similar trend in Blacks).

Results of the analysis of prospectively-ascertained *de novo* CVD are presented in Table 4. During 1,470 person-years of follow-up (median follow-up, 21 months), 141 *de novo* CVD events were recorded, with censoring for renal transplantation ($n=104$), and no losses to follow-up. Associations between lipoprotein(a) and CVD are stronger and have greater statistical significance for the ELISA at all cut-offs compared to the IT assay.

DISCUSSION

This study in the dialysis population compares the relative predictive value of an apolipoprotein (a)-size-independent ELISA assay compared to an apolipoprotein(a)-size-dependent IT assay in predicting CVD events and identifying individuals with LMW isoforms. The ELISA well out-performed the IT assay in both respects. Our results also characterize a novel interpretation for elevated lipoprotein(a) levels in dialysis patients, when measured with the ELISA, providing a clinically-available alternative to identify higher-risk individuals who have both high lipoprotein(a) levels and LMW isoforms. The linkage of these findings with the identification of an apolipoprotein(a) size-related bias in the IT assay levels (Figure 3) supports a potential mechanism (Figure 1) by which any apolipoprotein(a)-size-dependent assay might underestimate associations between lipoprotein(a) level and both CVD and LMW isoforms.

This hypothesis is supported by our results, which demonstrate a much stronger association between lipoprotein(a) level and prospective CVD when the ELISA is used, compared to the IT assay. These findings may explain some of the heterogeneity seen in studies of lipoprotein (a), since the vast majority of previously-published studies have used apolipoprotein(a)-sensitive assays of many types. Clinicians and researchers who assay lipoprotein(a) levels should know the characteristics of the assay they employ.

Predictive Value of Lipoprotein(a) Level in Identifying LMW Isoforms

We found that the apolipoprotein(a) size-insensitive ELISA accurately identifies individuals with LMW isoforms at lipoprotein(a) levels above the 85th percentile, with high specificity and PPV in both Blacks and Whites. However, at levels lower than the 85th percentile the ability to identify LMW isoforms falls off. The IT assay was not able to identify LMW isoforms at any cut-off. With regard to NPV (ability to “rule out” LMW isoforms), neither assay performs very well.

Distinguishing the relative importance of LMW apolipoprotein(a) size compared to elevated lipoprotein(a) level in predicting CVD events is challenging, since the two are so strongly associated, even in dialysis patients. Previous studies suggest that LMW apolipoprotein(a) isoforms promote atherogenesis through inhibition of fibrinolysis [15,16]. Apolipoprotein(a) has high degrees of homology with plasminogen [17] and LMW isoforms bind more strongly to fibrin than larger isoforms [18], suggesting that the LMW apolipoprotein(a) isoform size itself plays a role in atherogenesis. Furthermore, lipoprotein(a) usually accounts for <10% of LDL-cholesterol. Therefore if lipoprotein(a) acts only through its lipid portion, even a relatively large elevation in its level might have relatively small atherogenic effects.

In a dialysis cohort, we previously reported detailed data from the CHOICE Study [4] showing that high lipoprotein(a) concentration, when measured by the ELISA assay, prospectively predicts CVD (adjusted RH=1.88 for levels above the 90th percentile), as does LMW apolipoprotein(a) size (adjusted RH=1.96 for isoform size less than the 10th percentile). However, in that analysis, when both are modeled simultaneously, LMW apolipoprotein(a) size remains as a risk factor, but lipoprotein(a) level does not.

Whether it is the very high lipoprotein(a) levels or the LMW apolipoprotein(a) size or both that are important in atherogenesis, this study shows that the apolipoprotein(a) size-insensitive ELISA is able to identify, with a high degree of accuracy, the 15% of dialysis patients who have both LMW isoform size and high lipoprotein(a) levels. Although most medications do not effectively lower lipoprotein(a) levels, this group of individuals could potentially benefit from more aggressive CVD prevention efforts through reduction of other risk factors or by using a combination of a statin and niacin as an approach to lipid lowering.

Being able to identify individuals with *both* high lipoprotein(a) levels and a LMW isoform may also be clinically important, since some studies have reported a positive interaction between high lipoprotein(a) level and LMW isoform size in the risk of CVD outcomes in the general population [19,20].

A pattern has emerged in studies of lipoprotein(a) and CVD, finding that the risk of CVD increases most in individuals with highest levels of the lipoprotein(a) range, suggesting a possible “threshold” phenomenon [21]. Since lipoprotein(a) level can identify LMW isoforms only at the highest levels (as this study demonstrates) this would explain such a “threshold” phenomenon, suggesting that isoform size itself may be more important than lipoprotein(a) level. Even if this is the case, very high lipoprotein(a) levels may still be clinically useful as a marker for LMW isoforms, in the absence of a commercially available test for apolipoprotein (a) isoform size.

Comparison of the ELISA and IT Assays

A lipoprotein(a) concentration over the 85th percentile, as measured by the ELISA, predicted incident CVD and was able to accurately identify LMW isoforms. Conversely, the IT assay was not significantly associated with incident CVD and was not diagnostically useful in identifying LMW isoforms at any level.

A possible explanation for this finding is seen in Figure 3, which demonstrates a graded bias in the ratio of the two assay results according to apolipoprotein(a) size. In the introduction, a mechanism was presented by which such findings could be at least partially explained (Figure 1). Our data support the hypothesis that assays of lipoprotein(a) mass tend to underestimate lipoprotein(a) concentration among those with LMW isoforms and overestimate lipoprotein(a) concentration among those with HMW isoforms, leading to a weakened association between the two. Such biases, which would alter the lipoprotein(a) level-apolipoprotein(a) size relationship towards the null, have potential to explain the inability of the IT assay, at any cutoff, to accurately identify individuals with LMW isoforms. Also supporting this hypothesis is our finding that the ratios of lipoprotein(a) values from the lowest to highest apolipoprotein(a) quartiles were approximately twice as high for the ELISA than the IT assay in both Blacks and Whites (Table 3).

From a risk-stratification standpoint, this mechanism would also bias associations between lipoprotein(a) level and CVD towards the null, if some of the CVD risk of lipoprotein(a) is mediated through LMW isoforms. This study demonstrates such a bias, finding stronger, more statistically significant, associations between CVD and lipoprotein(a) when measured with ELISA, and weaker, statistically non-significant associations when the IT assay is used.

Importantly, a similar result was also reported by the Physicians' Health Study. They reported a strong association between lipoprotein(a) level >158.8 nmol/L and incident angina pectoris, using the same apolipoprotein(a) size-independent ELISA and laboratory we used ($RR=2.6$; $p\text{-trend}=0.02$; fifth versus first quartile), but a weaker non-significant association when an apolipoprotein(a) size-dependent nephelometric assay was used ($RR=1.9$, $p\text{-trend}=0.11$) [21]. To our knowledge, these are the only two studies reporting a head-to-head comparison of an apolipoprotein(a) size-sensitive versus size-insensitive assay and their prospective associations with CVD.

Replication of a similar bias in two different populations using the same apolipoprotein(a) size-insensitive assay and two different apolipoprotein(a) size-dependent assays increases the probability that it is the apolipoprotein(a) size-sensitivity, not some other characteristic of the IT or nephelometric assays that may be responsible for the weaker associations that have been found. The hypothesized bias also has potential to explain in part some of the highly disparate results seen in studies of lipoprotein(a) and CVD, almost all of which have used a wide variety of apolipoprotein(a) size-sensitive assays.

We caution against generalizing these inferences to all other apolipoprotein(a) size-sensitive assays not yet evaluated in research studies. However, sufficient evidence exists to warrant further investigation of the hypothesized bias, and its potential impact on inferences in studies using size-sensitive lipoprotein(a) assays.

These results support the conclusions of the Report of the National Heart, Lung, and Blood Institute Workshop on Lipoprotein(a) and CVD [5]. To compare data among different studies and to be able to correctly interpret the clinical usefulness of lipoprotein(a) data, the workshop recommended that assays for measuring lipoprotein(a) in studies be validated for their ability to provide accurate values independent of apolipoprotein(a) size.

Regarding limitations of the study, it is recognized that the storage time was about 20 months longer for the ELISA assay samples than for the IT assay. However, all study specimens were drawn, centrifuged, divided into aliquots, and handled in the same manner until freezing at -80°C . Assays for both tests were performed on previously non-thawed aliquots. The IT aliquots were thawed and assayed on-site at the DCI laboratory, whereas the previously non-thawed aliquots sent for the ELISA assay were sent on ice about 20 months later to the Northwest laboratory. Therefore, any sample degradation bias due to storage or handling differences (if present) would have degraded the ELISA samples more than the IT samples and biased our results in the opposite direction than observed. We therefore conclude that these minor differences cannot account for the significant bias seen in our analysis.

Conclusion

High lipoprotein(a) levels, when measured by an apolipoprotein(a) size-insensitive ELISA assay, strongly predict incident CVD and accurately identify LMW isoforms. An apolipoprotein(a) size-sensitive assay resulted in much weaker and statistically non-significant associations with CVD events and could not identify LMW isoforms at any level. This appeared to be related to a bias in the IT assay results related to apolipoprotein(a) size.

If such a bias is present for other apolipoprotein(a) size-sensitive assays, physicians and researchers should assess the clinical and research implications of the lipoprotein(a) assay method used.

This study also presents a novel clinical interpretation of lipoprotein(a) concentration among dialysis patients, by identifying a lipoprotein(a) concentration threshold that identifies individuals with both high lipoprotein(a) level and LMW apolipoprotein(a) size. Patients identified in this manner may potentially benefit from more aggressive CVD risk factor reduction and control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations

CV, Coefficient of variation
 ROC, Receiver-Operator Characteristics
 CVD/CHD, Cardiovascular disease/Coronary heart disease
 DCI, Dialysis Clinic, Incorporated
 ELISA, Enzyme-linked Immunosorbant Assay
 K-IV, Kringle-IV

HR, Hazard Ratio
 IT, immunoturbidometric
 LWM/HMW, Low/High molecular weight
 PPV/NPV, Positive/negative predictive value
 USRDS, United States Renal Data System

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Panel A: Apo(a) size-sensitive assay (using antibodies to repeating K-IV subunits)

- Repeating subunit
● Non-repeating subunit

Panel B: Apo(a) size-insensitive assay (using antibodies to a non-repeating K-IV subunit)

Figure 1. potential mechanism of bias in the determination of lipoprotein(a) level using an apolipoprotein (a) size-sensitive assay.

Footnote for Figure 1. Illustration of potential mechanism of bias in the determination of lipoprotein(a) concentration using an assay that measures lipoprotein(a) mass. Panel A demonstrates that even if the lipoprotein(a) concentration is the same in the reference sample and test samples, an apolipoprotein(a) size-sensitive lipoprotein(a) mass assay in an individual with LMW isoforms will underestimate the lipoprotein(a) concentration, and overestimate it in an individual with HMW isoforms. On the other hand, as shown in Panel B, the apolipoprotein(a) size-insensitive assay measures concentration accurately, and the signal is not affected or biased by the size of apolipoprotein(a).

Figure 2.
ROC Curve for the ELISA and IT assays, in identification of LMW apolipoprotein(a) isoforms.

Figure 3. Ratio of IT assay values to ELISA values, stratified by race and apolipoprotein(a) size category demonstrating a bias in the ratio of the IT assay to the ELISA, according to apolipoprotein size.
Footnote for Figure 3. *Non-parametric test for trend for the difference in median ratio across apo(a) groups.

Table 1

Operating characteristics of lipoprotein(a) in identification of LMW apolipoprotein(a) isoforms, by race and assay type in the CHOICE dialysis cohort

Lp(a) percentile cut-off	Lp(a) Level Cutoff Value	Sensitivity (%)		Specificity (%)		Positive Predictive Value for LMW size (%)		Negative Predictive Value for LMW size (%)	
		Estimate	95% C.I.	Estimate	95% C.I.	Estimate	95% C.I.	Estimate	95% C.I.
WHITES									
ELISA Assay									
15 %ile	3.8 nmol/l	90	[84, 94]	17	[13, 23]	43	[38, 48]	71	[57, 81]
25 %ile	10.6 nmol/L	83	[76, 88]	29	[24, 36]	45	[40, 51]	71	[61, 79]
75 %ile	105.6 nmol/L	49	[42, 57]	93	[89, 96]	83	[75, 90]	72	[67, 77]
85 %ile	158.8 nmol/L	34	[27, 42]	99.6	[98, 100]	98	[91, 100]	68	[63, 73]
Immunoturbidimetric Assay									
15 %ile	1.2 mg/dl	86	[80, 91]	16	[12, 21]	42	[37, 47]	63	[50, 74]
25 %ile	5.7 mg/dl	78	[71, 84]	27	[21, 33]	43	[37, 48]	63	[53, 72]
75 %ile	53.9 mg/dl	41	[33, 48]	85	[80, 90]	66	[56, 75]	67	[62, 72]
85 %ile	71.0 mg/dl	28	[22, 35]	94	[90, 96]	75	[63, 85]	65	[60, 70]
BLACKS									
ELISA Assay									
15 %ile	28.1 nmol/L	95	[89, 98]	22	[15, 30]	50	[44, 57]	84	[68, 94]
25 %ile	47.5 nmol/L	90	[83, 95]	37	[29, 45]	54	[47, 61]	81	[70, 90]
75 %ile	148.8 nmol/L	45	[36, 55]	92	[86, 96]	82	[70, 90]	67	[60, 73]
85 %ile	169.4 nmol/L	30	[22, 39]	97	[93, 99]	90	[76, 97]	62	[56, 69]
Immunoturbidimetric Assay									
15 %ile	18.6 mg/dl	91	[83, 96]	20	[13, 30]	50	[42, 58]	73	[52, 88]
25 %ile	28.8 mg/dl	86	[77, 93]	38	[28, 48]	55	[45, 64]	76	[61, 87]
75 %ile	90.0 mg/dl	41	[30, 52]	83	[74, 89]	67	[53, 80]	62	[52, 70]
85 %ile	129.9 mg/dl	23	[15, 34]	90	[82, 95]	68	[48, 95]	57	[49, 66]

Table 2
Diagnostic likelihood ratios and post-test probabilities for lipoprotein(a) level in the identification of LMW apolipoprotein(a) isoforms, by race and assay type.

Lpa(a) Percentile Groups	Pre-test Probability	Diagnostic Likelihood Ratio		Post-test Probability of LMW apolipoprotein(a) size	
		Estimate	95% C.I.	Estimate	95% C.I.
WHITES					
ELISA Assay					
0.0–15.0	0.41	0.6	[0.3, 1.1]	0.29	[0.17, 0.43]
15.1–25.0	0.41	0.6	[0.3, 1.2]	0.28	[0.15, 0.45]
25.1–75.0	0.41	0.5	[0.4, 0.8]	0.26	[0.20, 0.34]
75.1–85.0	0.41	2.9	[1.4, 6.2]	0.66	[0.49, 0.81]
> 85	0.41	28.5	[9.0, 114]	0.95	[0.86, 0.99]
Immunoturbidometric Assay					
0.0–15.0	0.41	0.9	[0.4, 1.5]	0.37	[0.22, 0.51]
15.1–25.0	0.41	0.8	[0.4, 1.6]	0.34	[0.20, 0.52]
25.1–75.0	0.41	0.6	[0.4, 0.9]	0.30	[0.30, 0.38]
75.1–85.0	0.41	1.6	[0.9, 3.1]	0.52	[0.35, 0.68]
> 85	0.41	4.4	[2.3, 8.5]	0.75	[0.62, 0.85]
BLACKS					
ELISA Assay					
0.0–15.0	0.46	0.2	[0.03, 0.5]	0.11	[0.02, 0.31]
15.1–25.0	0.46	0.4	[0.1, 1.3]	0.26	[0.08, 0.52]
25.1–75.0	0.46	0.9	[0.5, 1.5]	0.43	[0.30, 0.56]
75.1–85.0	0.46	1.4	[0.5, 4.4]	0.54	[0.30, 0.79]
> 85	0.46	14.9	[3.5, 133]	0.93	[0.75, 0.99]
Immunoturbidometric Assay					
0.0–15.0	0.46	0.4	[0.1, 1.1]	0.26	[0.11, 0.48]
15.1–25.0	0.46	0.3	[0.07, 0.9]	0.19	[0.06, 0.44]
25.1–75.0	0.46	1.0	[0.6, 1.8]	0.46	[0.32, 0.60]
75.1–85.0	0.46	2.3	[0.8, 7.0]	0.66	[0.40, 0.85]
> 85	0.46	2.4	[1.0, 6.4]	0.67	[0.45, 0.84]

Table 3
Comparison of median lipoprotein(a) levels using the ELISA and IT assays according to apolipoprotein(a) quartile, stratified by race

Assay	Apo(a) Quartile				p-value	Ratio of Median Lp(a)	
	1	2	3	4		Lowest to highest apo(a) quartile	
Whites							
ELISA (nmol/L)	159.4	20.3	45.6	16.0	<0.0001	10.0	
IT (mg/dl)	61.2	11.2	27.2	13.9	<0.0001	4.4	
Blacks							
ELISA (nmol/L)	160.0	108.1	64.1	26.3	<0.0001	6.1	
IT (mg/dl)	75.1	55.5	40.4	20.2	<0.0001	3.7	

Table 4
Adjusted associations between lipoprotein(a) level and prospective *de novo* CVD events, by assay type:

Lipoprotein(a) Groups	ELISA Assay			IT Assay		
	Adjusted HR [†]	95% C.I.	P	Adjusted HR [†]	95% C.I.	P
Lp(a) Model 1						
<85 th Percentile	1.0	Reference [1.0, 3.3]	0.045	1.00	Reference [0.7, 2.4]	0.37
≥85 th Percentile	1.8			1.3		
Lp(a) Model 2						
<50 th Percentile	1.0	Reference [0.8, 1.9]	0.43	1.0	Reference [0.7, 1.8]	0.69
50 th – 84 th Percentile	1.2		0.03	1.1		0.33
≥85 th Percentile	2.0	[1.1, 3.7]	0.04	1.4	[0.7, 2.6]	0.34
<i>p-trend</i>						

[†] All four Cox Proportional Hazards models of survival to *de novo* CVD event outcomes were adjusted for age, race, sex, dialysis modality, baseline CVD, comorbidity score, cause of renal disease, total cholesterol, HDL-cholesterol quartile, smoking status, and systolic blood pressure quartile, albumin, creatinine, BMI quartiles, and C-reactive protein