Small Apolipoprotein(a) Size Predicts Mortality in End-Stage Renal Disease

The CHOICE Study

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Background—The high mortality rate in end-stage renal disease has engendered interest in nontraditional atherosclerotic cardiovascular disease (ASCVD) risk factors that are more prevalent in end-stage renal disease, such as elevated lipoprotein(a) [Lp(a)] levels. Previous studies suggest that high Lp(a) levels and small apolipoprotein(a) [apo(a)] isoform size are associated with ASCVD, but none have investigated the relationship between Lp(a) level, apo(a) size, and mortality.

Methods and Results—An inception cohort of 864 incident dialysis patients was followed prospectively. Lp(a) was measured by an apo(a) size-independent ELISA and apo(a) size by Western blot after SDS-agarose gel electrophoresis. Comorbid conditions were determined by medical record review. Time to death was ascertained through dialysis clinic and Health Care Financing Administration follow-up. Survival analyses were performed with adjustment for baseline demographic, comorbid conditions, albumin, and lipids. Median follow-up was 33.7 months, with 346 deaths, 162 transplantations, and 10 losses to follow-up during 1999 person-years of follow-up. Cox regression analysis showed no association between Lp(a) level and mortality. However, an association between small apo(a) isoform size and mortality was found (hazard ratio, 1.36; P=0.004) after adjusting for age, race, sex, comorbidity score, cause of renal disease, and congestive heart failure. The association was somewhat lower in white patients (hazard ratio 1.34; P=0.019) than in black patients (1.69; P=0.04). No interaction by age, race, sex, diabetes, ASCVD, or Lp(a) level was present.

Conclusions—Small apo(a) size, but not Lp(a) level, independently predicts total mortality risk in dialysis patients.

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Key Words: lipoproteins • mortality • kidney

The contribution of atherosclerotic cardiovascular disease (ASCVD) to the extraordinarily high mortality in end-stage renal disease (ESRD) has generated interest in nontraditional ASCVD risk factors that are prevalent in ESRD, such as lipoprotein(a) [Lp(a)]. Lp(a), an atherogenic lipoprotein, consists of an LDL-cholesterol particle covalently bonded to apolipoprotein(a) [apo(a)], a glycoprotein with a genetic-size polymorphism. Lp(a) level is strongly and negatively associated with apo(a) isoform size 4 and is elevated in dialysis patients, 5 suggesting that Lp(a) level or small apo(a) size may account for a portion of the increased ASCVD or mortality in the ESRD population.

Prospective studies of Lp(a) and ASCVD in ESRD have yielded conflicting results. 6–8 The largest study in this population to date found that incident ASCVD was associated with small apo(a) size but not with a high Lp(a) level, 6 and at least one prospective study found Lp(a) level not to be associated with total mortality among dialysis patients. 9 Because no previous studies, to our knowledge, have evaluated an association between apo(a) size and total mortality in any population, we tested the a priori hypothesis that elevated Lp(a) level and small apo(a) size predict mortality in a nationally representative cohort of patients beginning dialysis.

Methods

Study Design and Population

The Choices for Healthy Outcomes in Caring for ESRD (CHOICE) study is a prospective study of outcomes among incident dialysis patients. From October 1995 to June 1998, 1041 participants from 19 states were enrolled from 81 dialysis clinics associated with Dialysis Clinic, Incorporated (DCI, Nashville, Tenn; n=923 from 79 clinics), New Haven CAPD (New Haven, Conn; n=86 from 1 clinic), or Saint Raphael’s Hospital (New Haven, Conn; n=32 from 1 clinic). Blood was drawn only from participants enrolled at the DCI clinics, of whom 864 (93.6%) had Lp(a) measured. Participants were recruited a median of 1.6 months after first dialysis (98% within 4
months). Enrollment criteria included initiation of dialysis in the preceding 3 months, ability to give written informed consent, age over 17 years, and ability to speak English or Spanish. Trained recruiters at each clinic presented the study to all new dialysis patients during the recruitment period. The Johns Hopkins University School of Medicine Institutional Review Board and the review boards for the clinical centers approved the protocol.

Data Collection

Serum was collected before a dialysis session, centrifuged, sent overnight to the DCI Central Laboratory, stored at -80 oC, and sent on dry ice to the Northwest Lipid Research Laboratory, University of Washington, Seattle, for Lp(a) assay. Sample draw occurred at a median of 4.4 months after first dialysis (95% within 6.8 months).

Lp(a) concentration was measured by a direct-binding double monochlonal antibody-based enzyme-linked immunosassay (ELISA), as previously reported. The detection antibody is directed to a nonrepeating epitope present in apo(a) K-IV type 9, making this assay insensitive to apo(a) size. Lp(a) concentrations were expressed in nanomoles per liter (nmol/L). The coefficient of variation (CV) of log-Lp(a) based on 49 blindly split samples from the CHOICE cohort was 6.8%.

Apo(a) isoforms were characterized using a high-resolution SDS-agarose gel electrophoresis method followed by immunoblotting, as previously reported. We used a size designation related to each isoform’s number of K-IV repeats. The CV for apo(a) size in the CHOICE cohort was 11.7% (n=49). 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%.

On a participant’s death, records and study forms are sent from the dialysis clinic to the data coordinating center, and vital status are verified actively every 3 months. For 107 of 117 individuals who left the study for reasons other than renal transplantation, vital status was passively determined using Health Care Financing Administration data.

Age, race, sex, and tobacco use were obtained from patient questionnaires. The average of all predialysis session systolic and diastolic blood pressures for the baseline 3-month period was determined for each participant (average, 31 blood pressure readings per participant). The prevalence of hypertension (blood pressure >140/90), diabetes, congestive heart failure (CHF), and cardiovascular disease (defined as any history of myocardial infarction, cardiac revascularization procedure, stroke, carotid endarterectomy, peripheral vascular disease or revascularization procedure, angina, or stress test positive for ischemia) was determined from review of dialysis clinic records by one of two dialysis research nurses. Mention of a condition (past or present) in the medical record was sufficient for positive coding. A validated composite baseline co-morbidity score (range, 0 to 3) derived from the Index of Co-Existent Disease (ICED) was calculated (Kappa=0.93, based on 45 charts blindly reviewed by both nurses).

Statistical Analysis

Statistical analyses were performed with Stata (version 6.0). The Mann-Whitney U test was used to test for differences in median values of skewed variables. All analyses of apo(a) size used the predominantly expressed isoform. Lp(a) was dichotomized at the median and log-transformed when analyzed continuously. Apo(a) size was dichotomized at 22 K-IV repeats. Both were also analyzed by quartile and tested for trend across quartiles.

Survival time was defined as time from exposure ascertainment (ie, blood draw) to death or censoring. The time scale used in the analysis was time from first dialysis, with staggered entry at time of exposure ascertainment. To allow for stable early survival estimates, entry was left-truncated at 4.0 months after first dialysis.

Of the 872 participants with Lp(a) measured, 8 were not eligible for inclusion in survival analyses because they had Lp(a) measured after withdrawal from the study or were closed out before the 4.0-month entry. Of the 864 analyzed, 346 (40.1%) died, 10 (1.1%) were lost to follow-up, 162 (18.8%) were censored on renal transplantation to avoid an informative censoring bias, and the remainder were censored administratively on November 1, 2000.

Several groups of covariates were selected a priori for inclusion in Cox regression models. Group 1 included baseline dialysis modality, age, race, and sex. Group 2 added the ICED score, cause of renal disease, and CHF (CHF was chosen over ASCVD because it predicted mortality more strongly than ASCVD). Group 3 added albumin, total cholesterol, HDL-cholesterol, smoking status, and systolic blood pressure. Substitution of diastolic blood pressure, mean arterial pressure, or pulse pressure for systolic blood pressure made negligible differences in the model estimates. A priori stratified analyses investigating interactions were also performed by race, sex, age, diabetes, ASCVD, Lp(a) level, and apo(a) size. No variables were found to violate the proportionality assumption of the Cox model.

Results

Cohort characteristics are described in Table 1. The age, sex, race, and dialysis modality distributions are similar to the 1997 incident United States dialysis population reported in the United States Renal Data System (USRDS, 1999 Report). The proportion on peritoneal dialysis (PD) is higher than USRDS because CHOICE over-sampled PD patients.

Median Lp(a) levels, stratified by race, did not differ by survival status. Median apo(a) size was significantly higher among black survivors compared with black nonsurvivors but similar among white survivors and nonsurvivors.

Cox regression models. Group 1 included baseline dialysis modality, age, race, and sex. Group 2 added the ICED score, cause of renal disease, and CHF (CHF was chosen over ASCVD because it predicted mortality more strongly than ASCVD). Group 3 added albumin, total cholesterol, HDL-cholesterol, smoking status, and systolic blood pressure. Substitution of diastolic blood pressure, mean arterial pressure, or pulse pressure for systolic blood pressure made negligible differences in the model estimates. A priori stratified analyses investigating interactions were also performed by race, sex, age, diabetes, ASCVD, Lp(a) level, and apo(a) size. No variables were found to violate the proportionality assumption of the Cox model.

The transplant group did not differ significantly by apo(a) subtype (25 versus 24 K-IV repeats; P=0.50) but did have a lower Lp(a) level (45 versus 55 nmol/L; P=0.05).

Figure 1 shows no trend in death rate by Lp(a) level but a significant negative trend across apo(a) size among black patients and a trend approaching significance across apo(a) size among white patients. No black patients had an apo(a) size <13 K-IV repeats, and only one black patient had an apo(a) size >36 K-IV repeats. Figure 2 demonstrates lower survival among white than black patients but no association with Lp(a) level within race strata. However, participants with low-molecular-weight (LMW) (<22 K-IV repeats) apo(a) size had statistically significant lower survival among black patients and a trend toward lower survival among white patients.

The association between LMW apo(a) size and mortality remained after adjustment for all 3 groups of covariates (Table 2). The association was similar in white and black patients, although the estimate for black patients was not statistically significant (P interaction by race=0.38). The test for trend across apo(a) size quartile was significant in all models. No interaction by age, race, sex, diabetes, prevalent cardiovascular disease (CVD), or Lp(a) level was present.
Discussion

An association between small apo(a) size and incident ASCVD has been previously described in ESRD, but to our knowledge, no studies have reported the relationship between apo(a) size and total mortality in any population. This study identifies small apo(a) size as a new and independent risk factor of moderate magnitude for total mortality in a cohort of incident dialysis patients but finds no association between Lp(a) level and mortality. This focus on total mortality is important particularly among dialysis patients, because the mortality in this population is so high. Furthermore, some ASCVD risk factors may not predict total mortality. Those that do, such as small apo(a) size in this study, may have greater public health impact than those that do not predict total mortality.

Previous Studies of Lp(a) and apo(a) in the General Population

The association between Lp(a) level and coronary heart disease (CHD) has been studied extensively in the general population. A meta-analysis of 18 prospective studies found an overall risk ratio of 1.7 (95% CI, 1.4 to 1.9) for fatal and nonfatal CHD comparing the upper and lower Lp(a) tertiles. The HERS trial reported similar relative risks of CHD in postmenopausal women, finding that estrogen replacement therapy significantly lowers Lp(a) level and confers a greater CHD protective benefit in those with high Lp(a) levels compared with those with low Lp(a).

Fewer prospective studies of apo(a) size and CHD in the general population have been published. The largest nested case-control study of Lp(a), apo(a), and incident CHD in the general population (n=110,051 cases) found an association between Lp(a) and CHD but not between apo(a) size and CHD, although the crude analysis showed a trend toward smaller apo(a) size among cases. The prospective studies of apo(a) size were not large enough to study mortality, and the largest studies of Lp(a) level, which might have had the power to analyze mortality, did not measure apo(a) size.

Potential Atherogenic Mechanisms of Lp(a) and apo(a)

The 80% sequence homology of the apo(a) K-IV moiety with plasminogen and in vitro findings that Lp(a) inhibits fibrino-
ysis suggests that Lp(a) may contribute to acute thrombotic events. Hervio et al reported that the LMW apo(a) isoforms bind more strongly to fibrin than larger isoforms. This provides a possible mechanism by which apo(a) isoform size itself, not only the associated Lp(a) level, becomes important in accelerating atherosclerosis, particularly after significant atherosclerosis has already been established. Nevertheless, in this mechanism, Lp(a) level would probably still be expected to play a significant role, because more apo(a) would be available to inhibit fibrinolysis, and an interaction between Lp(a) level and apo(a) size in the association with incident ASCVD or mortality could result.

Kronenberg and colleagues found an association between Lp(a) and progression of atherogenesis and incident CVD restricted to those with no baseline atherosclerosis. In contrast, they found an association between small apo(a) isoform size and progression to advanced atherosclerosis restricted to those with baseline atherosclerosis. The results of that study suggest that Lp(a) may act primarily as an atherogenic lipid (via its LDL-like portion) in vessels with little or no atherosclerosis, whereas in the setting of advanced atherosclerosis, the antifibrinolytic action of the apo(a) protein may play a more significant role.

Our finding of an association between small apo(a) size and mortality supports this proposed mechanism, because atherosclerosis is so highly prevalent in ESRD. Furthermore, although there was insufficient evidence of interaction by prevalent CVD (Table 3), we did find a higher risk of death for LMW isoforms among those with prevalent CVD than without.

**Lp(a) Level, apo(a) Size, and Mortality in ESRD**

The only prospective study of Lp(a) level, apo(a) size, and CHD in ESRD, which followed 440 dialysis patients for 5 years and accrued 66 CHD events, found that LMW apo(a) size, not Lp(a) level, was associated with the development of CHD (adjusted hazard ratio [HR] = 2.3; P = 0.0008). Kronenberg and colleagues have developed an interesting hypothesis based on their finding that among those with HMW apo(a) isoforms, Lp(a) levels are much higher in ESRD than in apo(a) phenotype-matched population controls, whereas among those with LMW isoforms, the Lp(a) level in ESRD patients is not significantly higher than that of phenotype-matched population controls, who already have high Lp(a) levels. This implied differential change in Lp(a) by apo(a) subtype over the course of progression to renal disease would diminish the association between Lp(a) and ASCVD in ESRD. Furthermore, the hypothesis states that in dialysis patients, apo(a) isoform size would more accurately reflect Lp(a) levels actually experienced over the long term, identify those with a more damaged vascular endothelium at the beginning of ESRD, and therefore possibly identify those who would develop ASCVD at a much higher rate.

On the other hand, apo(a) size remains the strongest predictor of Lp(a) levels even after ESRD develops. In our cohort, apo(a) size alone accounts for ~38% of the variability.
in Lp(a) level. It should not be expected, therefore, that the processes in ESRD would completely remove the association between Lp(a) and ASCVD. The present study found no association between Lp(a) level and mortality. These findings are consistent with the study by Koch et al., who measured Lp(a) level but not apo(a) size in 130 dialysis patients followed for 36 months, finding no association between Lp(a) level and survival. Several potential explanations exist for the null finding for Lp(a) level in light of the positive association with apo(a) size. First, our results are consistent with Kronenberg’s hypothesis, which would predict a stronger association between apo(a) size and mortality in patients with ESRD.

Figure 2. Unadjusted Kaplan-Meier plots of survival by race, Lp(a) level (A), and apo(a) size (B).

### Table 2. Adjusted Associations Between LMW apo(a) Size and Mortality by Adjustment Group and Race

<table>
<thead>
<tr>
<th>Model Population</th>
<th>Adjustment Group</th>
<th>Model, n</th>
<th>Deaths, n</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
<th>Adjusted HR of Death Apo(a) ≤22 K-IV vs ≥23 K-IV Repeats</th>
<th>Adjusted HR of Death, by Quartile of apo(a) size*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>Group 1†</td>
<td>864</td>
<td>346</td>
<td>1.33</td>
<td>[1.08, 1.65]</td>
<td>0.008</td>
<td>1.29</td>
<td>1.18</td>
</tr>
<tr>
<td>All</td>
<td>Group 2‡</td>
<td>864</td>
<td>346</td>
<td>1.36</td>
<td>[1.10, 1.69]</td>
<td>0.004</td>
<td>1.38</td>
<td>1.26</td>
</tr>
<tr>
<td>All</td>
<td>Group 3§</td>
<td>754</td>
<td>300</td>
<td>1.41</td>
<td>[1.11, 1.77]</td>
<td>0.004</td>
<td>1.51</td>
<td>1.27</td>
</tr>
<tr>
<td>White</td>
<td>Group 2‡</td>
<td>562</td>
<td>261</td>
<td>1.34</td>
<td>[1.05, 1.72]</td>
<td>0.019</td>
<td>1.38</td>
<td>1.31</td>
</tr>
<tr>
<td>Black</td>
<td>Group 2‡</td>
<td>258</td>
<td>70</td>
<td>1.69</td>
<td>[1.04, 2.75]</td>
<td>0.04</td>
<td>2.25</td>
<td>1.30</td>
</tr>
</tbody>
</table>

*apo(a) quartile values: Q1, 0–18 K-IV repeats; Q2, 19–23 K-IV repeats; Q3, 24–28 K-IV repeats; and Q4, 29–40 K-IV repeats (reference group).
†Adjusted for age, race (black, white, other), and sex.
‡Adjusted for group 1 plus comorbidity score, cause of renal disease, and congestive heart failure.
§Adjusted for group 2 plus albumin quartile, total and HDL-cholesterol quartile, smoking status, and systolic blood pressure quartile.
P<0.05.
ASCVD (and, by extension, total mortality) than between Lp(a) levels and mortality for the reasons stated above.

A second potential reason for the null Lp(a) finding is informative censoring associated with transplantation. Lp(a) levels were higher among the adherent group than among the healthier, younger, transplanted group. This selective removal of healthy patients with lower Lp(a) levels could have diminished the association of Lp(a) with mortality, if it were present.

Although the mortality risk associated with LMW apo(a) size is moderate, it may provide clinical usefulness in risk stratification or identification of dialysis patients who warrant more aggressive therapy with statins or other Lp(a)-lowering therapies. These findings raise the question of whether measurement of Lp(a) level alone among dialysis patients is useful, because it does not predict total mortality. If the risk of ASCVD related to Lp(a) is mediated by both apo(a) isoform size and the attendant Lp(a) level, then Lp(a)-lowering therapy has the potential to prevent deaths, particularly among those with LMW apo(a) isoforms. However, if the primary atherogenic effect of Lp(a) is mediated not through total Lp(a) level but through genetic mechanisms that determine LMW apo(a) isoforms, then reducing Lp(a) levels of HMW isoforms may not produce a benefit. In this case, therapies specifically directed against the action of LMW isoforms may be most effective. Future studies should explore whether the risk associated with LMW isoforms is attributable to their correlation with long-term elevation in Lp(a) levels or unique DNA variations in linkage disequilibrium with LMW isoforms, which could explain a heterogeneity in risk associated with different Lp(a) particles.

The strengths of this study include the representative national sample of participants, enrollment on initiation of dialysis, the high follow-up rate, and the reliability of the apo(a) and Lp(a) assays. The most important limitation of this study is the high transplantation rate (18.8%), which could lead to informative censoring and bias, particularly for the Lp(a) results (discussed above). However, informative censoring is probably not a major issue for the apo(a) size findings, because the distribution of apo(a) size did not differ by transplantation status.

**Summary**

In summary, this study reports a new, independent association between LMW apo(a) isoforms and mortality of moderate magnitude in a large, nationally representative cohort of incident dialysis patients but no association between Lp(a) level and mortality. The lack of association between a single measurement of Lp(a) and the risk of mortality suggests that such a simplified approach may be inadequate for mortality risk stratification in dialysis patients. Studies of apo(a) size and mortality should be encouraged in some of the large prospective cohort studies in the general population that

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**TABLE 3. Adjusted Associations Between Lp(a) Level, Apo(a) Size, and Mortality Stratified by A Priori Subgroups**

<table>
<thead>
<tr>
<th>Stratified Models, by Group</th>
<th>Model, n Deaths, n</th>
<th>HR 95% CI</th>
<th>P</th>
<th>$P_{interaction}$ for Differences in Subgroup HRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>864 346</td>
<td>1.36 [1.10 to 1.69]</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Age &lt;60 y</td>
<td>433 117</td>
<td>1.51 [1.05 to 2.20]</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>≥60 y</td>
<td>431 229</td>
<td>1.36 [1.04 to 1.77]</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Sex Female</td>
<td>403 165</td>
<td>1.53 [1.12 to 2.10]</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>461 181</td>
<td>1.21 [0.90 to 1.63]</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>History of CVD No</td>
<td>372 90</td>
<td>1.07 [0.70 to 1.64]</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>492 256</td>
<td>1.44 [1.12 to 1.85]</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Diabetes No</td>
<td>387 129</td>
<td>1.45 [1.01 to 2.06]</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>477 217</td>
<td>1.32 [1.00 to 1.74]</td>
<td>0.046</td>
<td></td>
</tr>
<tr>
<td>Lp(a) &lt;53.0 nmol/L</td>
<td>433 187</td>
<td>1.75 [1.27 to 2.43]</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>≥53.0 nmol/L</td>
<td>431 158</td>
<td>1.44 [1.04 to 2.00]</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for age, race, sex, comorbidity score, cause of renal disease, and congestive heart failure.

†CVD defined as any history of myocardial infarction, cardiac revascularization procedure, stroke, carotid endarterectomy, peripheral vascular disease or revascularization procedure, angina, or stress test positive for ischemia.
have serum banked and have long follow-up periods and sufficient deaths already accrued for analysis.

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References

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