Lipoprotein(a) Level Does Not Predict Restenosis After Percutaneous Transluminal Coronary Angioplasty

Poonam Alaigh, Carol J. Hoffman, Giridhar Korlipara, Arlene Neuroth, John P. Dervan, William E. Lawson, Mae B. Hultin

Abstract—The serum lipoprotein(a) [Lp(a)] level is a known risk factor for arteriosclerotic coronary artery disease. However, its association with restenosis after percutaneous transluminal coronary angioplasty (PTCA) is controversial. We hypothesized that the Lp(a) level is a significant risk factor for restenosis after angioplasty through a pathophysiological mechanism leading to excess thrombin generation or inhibition of fibrinolysis. We designed a prospective study of the relation of Lp(a) to outcome after PTCA, in which we measured selected laboratory variables at entry and collected clinical, procedural, lesion-related, and outcome data pertaining to restenosis. Restenosis was defined as >50% stenosis of the target lesion by angiography or as ischemia in the target vessel distribution by radionuclide-perfusion scan. Before the patients underwent PTCA, blood was obtained by venipuncture for measurement of Lp(a), total cholesterol, thrombin-antithrombin (TAT) complex, α₂-antiplasmin–plasmin (APP) complex, and plasminogen activator inhibitor-1 (PAI-1). Evaluable outcome data were obtained on 162 subjects, who form the basis of this report. Restenosis occurred in 61 subjects (38%). The Lp(a) level was not correlated significantly with TAT, APP, PAI-1, or the TAT-APP ratio. Levels of TAT, APP, and PAI-1 were not statistically different in the patients with versus those without restenosis. The median ratio of TAT to APP was 2-fold higher in the restenosis group, and this difference approached statistical significance (P=0.07). Univariate analysis was performed for the association of clinical, lesion-related, and procedural risk factors with restenosis. Lp(a) levels did not differ significantly in the restenosis versus no-restenosis group, whether assessed categorically (>25 mg/dL versus <25 mg/dL) or as a continuous variable by Mann-Whitney U test. The number of lesions dilated and the lack of family history of premature heart disease were significantly associated with restenosis (P=0.002 and P=0.008, respectively). A history of diabetes mellitus was of borderline significance (P=0.055). By multiple logistic regression analysis, the number of lesions dilated was the only variable significantly associated with restenosis (P=0.03). We conclude that the number of lesions dilated during PTCA is a significant risk factor for restenosis, whereas the serum Lp(a) level was not a significant risk factor for restenosis in our patient population. The TAT to APP ratio merits further study as a possible risk factor for restenosis. (Arterioscler Thromb Vasc Biol. 1998;18:1281-1286.)

Key Words: lipoprotein(a) ■ angioplasty ■ thrombin ■ plasmin ■ antiplasmin

Percutaneous transluminal coronary angioplasty (PTCA) has become a standard procedure for the treatment of coronary artery disease. However, in spite of advances in techniques, restenosis after PTCA remains a common complication, at a rate of 30% to 40% in the absence of stent placement. Various risk factors predictive of restenosis have been identified, and these can be categorized as clinical, lesion-related, or procedural. The role of lipoprotein(a) [Lp(a)] as a risk factor for restenosis is controversial, although it is a proven risk factor for atherosclerosis. Some studies found the Lp(a) level to be a predictor for restenosis, whereas other studies showed no significant correlation of the Lp(a) level with restenosis. Most of those studies were either partly or completely retrospective, based on assays of stored blood samples, or included subjects with recent myocardial infarction. These are all factors in study design that are capable of confounding data collection and interpretation.

Control of the Lp(a) level is genetically transmitted in an autosomal dominant mode. The total content of Lp(a) in blood represents a class of lipoproteins that are heterogeneous both in size and density, but all contain apo(a) linked to apoB-100 by a disulfide bond. Lp(a) may inhibit fibrinolysis because of the sequence homology of apo(a) to plasminogen, may enhance atherogenesis by interfering with the normal uptake of apoB-100-containing particles by the LDL receptor, or may promote vascular smooth muscle cell proliferation by inhibiting transforming growth factor-β. The purpose of this prospective cohort study was 2-fold: (1) to determine whether the Lp(a) level measured before

Received September 18, 1997; revision accepted February 26, 1998.
From the Department of Medicine, State University at New York at Stony Brook.
Reprint requests to Mae B. Hultin, MD, University at Stony Brook Division of Hematology, Health Sciences Center T15-040, Stony Brook, NY 11794-8151. E-mail Mhultin@mail.som.sunysb.edu
Preliminary results of the study were published in abstract form (Blood. 1995;86:372a and Thromb Haemost. 1997;[suppl I]:720).
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PTCA is predictive of restenosis and (2) to determine whether the Lp(a) level influences the extent of thrombin generation or fibrinolytic activity. Thrombin-antithrombin (TAT) complex was measured as a marker of in vivo thrombin generation, and α2-antiplasmin–plasmin (APP) complex and plasminogen activator inhibitor 1 (PAI-1) antigen were measured as markers of fibrinolytic activity. The TAT to APP ratio was calculated as a measure of the imbalance between thrombin and plasmin activity.

### Methods

The study protocol was approved by the Committee on Research Involving Human Subjects at the State University of New York at Stony Brook, according to the principles of the Declaration of Helsinki. Written, informed consent was obtained from all participants. All patients referred for possible PTCA between August 1993 and April 1996, except for a few months’ hiatus in 1995, were considered eligible for possible enrollment. Exclusion criteria were myocardial infarction within the previous 6 weeks and hemodynamic instability before enrollment, and enrolled subjects who underwent stent placement in addition to PTCA were also excluded. The study had 3 parts.

In part 1, subjects were interviewed before undergoing PTCA. Data were collected regarding each subject’s demographics, history of clinical risk factors for restenosis, and medication use. Blood samples were collected from the subjects’ cardiologists and were reviewed by another cardiologist who was blinded to the Lp(a) results. Restenosis was defined as ≥50% stenosis of the target lesion or ischemia in the target vessel distribution as demonstrated by radionuclide-perfusion scan. Data were stored in database format (DataEase software, DataEase International).

### Blood Samples

The first tubes of blood were collected by venipuncture into sterile Vacutainer tubes (Becton Dickinson Systems) and allowed to clot. Serum was prepared by centrifugation for determination of serum total cholesterol and Lp(a). Subsequent tubes of blood were drawn into Stago Diatube H tubes containing sodium citrate, citric acid, theophylline, adenosine, and dipryridamole (American Bioproducts Company) for plasma PAI-1 determination and into SCAT-1 tubes containing EDTA, PPACK thrombin inhibitor, and aprotinin (Hematologic Technologies) for plasma TAT complex and αAPP complex determinations. Plasma was prepared by centrifugation at 3000g for 18 minutes at room temperature, divided into small (<1-mL) aliquots, stored at −80°C, and assayed within 1 month.

### Assays

Serum Lp(a) was measured by nephelometry at Specialty Laboratories Inc with goat polyclonal antibodies directed against apol(a). The normal range was 0 to 25 mg/dL. Lp(a) determination was performed on fresh samples within 1 week of blood collection. Quantification by this method was chosen to minimize the influence of isoform variations of Lp(a). The intra-assay coefficient of variation (CV) was 2%, and the interassay CV was <9.8%. Serum total cholesterol levels were assayed on fresh samples with DART reagents on a DACOS analyzer (Coulter Diagnostics) Plasma PAI-1 levels were measured by enzyme immunoassay (Biopool TintElize PAI-1) according to the manufacturer’s directions. Plasma TAT and APP complexes were measured by enzyme immunoassay (Enzygnost TAT micro and Enzygnost EIA APP micro, Behring Diagnostics) according to the manufacturer’s directions. To establish a normal range for TAT, APP, and PAI-1 values in our laboratory, a group of 32 healthy volunteers (17 women and 15 men) on no medications was recruited. Samples were drawn in the same manner as for the subjects undergoing PTCA. Fasting serum total cholesterol was also measured on 25 of the 32 subjects. The normal means or medians and ranges are summarized in Table 1. The mean fasting serum cholesterol level was 198±28 mg/dL. The median TAT level was 0.99 µg/L (range, 0 to 9.8), mean APP level was 295 µg/L (median=273; range, 90 to 660), and mean PAI-1 level was 7.9 µg/L (median=6.5; range, 0 to 23). These values are in good agreement with the manufacturer’s median reference value of 1.5 µg/L (range, 1.0 to 4.1) for the TAT assay; mean reference value of 210±88 µg/L (ranges 80 to 470) for the APP assay; and mean reference value of 18±10 ng/mL (range, 4 to 43) for the PAI-1 assay. The intra-assay CVs were 4% to 6%, 5% to 7%, and 2% to 7% for the TAT, APP, and PAI-1 assays, respectively. The interassay CV was 6% to 9% and 6% to 8% for the TAT and APP assays, according to the manufacturers. TAT levels can be falsely elevated owing to ex vivo generation of thrombin; measures to minimize this possibility included the use of the inhibitor mix in SCAT-1 tubes and the avoidance of traumatic or difficult venipuncture.

### Statistical Analysis

Data analyses were performed on an IBM personal computer with CSS Statistica software (Statsoft). Observed distributions were analyzed for departure from normality by χ² and Kolmogorov-Smirnov d statistics. Correlations of plasma PAI-1, TAT, APP, or...
TAT-APP ratio with serum Lp(a) levels were analyzed by Spearman’s rho statistics. For the univariate analyses, differences between TAT, PAI-1, TAT-APP ratio, or Lp(a) levels in the restenosis versus no-restenosis group were analyzed by the Mann-Whitney U test. Unpaired Student’s t tests were performed for other continuous variables (age and cholesterol) that were normally distributed. χ² tests were performed for categorical variables. Multiple logistic regression analysis was performed to determine independent predictors of restenosis.

Results

Markers of Thrombin Generation and Fibrinolysis

Seventy-three subject samples in the first half of our study were assayed for TAT, APP, and PAI-1. Results are summarized in Table 1. This group was composed of 54 men and 19 women with a mean ± SD age of 58.5 ± 10 years. Clinical restenosis was documented in 28 patients (38%). Median Lp(a) level was 24 mg/dL (range, 1 to 91) for the restenosis group and 13 mg/dL (range, 0 to 170) for the no-restenosis group. This difference in Lp(a) levels between the 2 groups was not statistically significant (P = 0.39) by the Mann-Whitney U test. Levels of TAT, APP, and PAI-1 also did not differ significantly between subjects with and without clinical restenosis (P = 0.13, 0.88, and 0.83, respectively). However, the median ratio of TAT to APP was ~2-fold higher in the restenosis group compared with the no-restenosis group (5.4 versus 2.9), and this difference approached statistical significance (P = 0.07). Some of the high TAT levels in the patient groups (eg, >10) could theoretically be related to difficult venipuncture, but the difference in the median values of the TAT-APP ratio between the restenosis and no-restenosis groups is not likely to have been caused by such outlier values, which were found in both groups. The median TAT-APP value in the normal subjects was less than in the restenosis group but more than in the no-restenosis group. Lp(a) levels were not correlated significantly with TAT, APP, PAI-1, or the TAT-APP ratio (P = 0.87, 0.49, 0.56, and 0.95, respectively).

Clinical and Procedural Variables: Relation to Restenosis After PTCA

All patients presenting for cardiac catheterization and possible angioplasty in the interval 1993 to 1996 were potentially eligible for enrollment in this cohort study. Of 269 subjects who consented to participate, 187 subjects had a successful angioplasty and completed parts 1 and 2 of the study; of these, 25 subjects did not undergo objective postprocedural evaluation at 4 to 6 months to determine the presence of restenosis. These 25 were excluded from the present data analysis. One hundred sixty-two subjects who had evaluable follow-up data (117 men and 45 women) and entry Lp(a) levels form the basis of this report; 23 subjects had subsequent coronary angiography, and 116 subjects had either exercise treadmill testing or intravenous dipyridamole in conjunction with thallium-201 scintigraphy. Patients with >50% stenosis of the treated lesion by angiography or ischemia in the target vessel distribution by thallium scan were classified as having clinical restenosis (n = 58). Of the 23 subjects who underwent exercise treadmill testing only, those with a positive test (n = 3) were classified as having clinical restenosis. Table 2 summarizes the clinical characteristics of the entire 162-subject study group. There were no significant differences in age, sex, number of vessels dilated, medication usage, smoking, clinical history of hypertension, or previous myocardial infarction by univariate analysis for the subjects in the restenosis group versus those in the no-restenosis group. Because >90% of the study subjects were white, subset analysis by race was not performed. Total serum cholesterol levels (available on 110 subjects) and Lp(a) levels also did not differ significantly between the 2 groups (P = 0.79 and 0.31, respectively). When Lp(a) was analyzed categorically, 31 of 73 subjects with an Lp(a) level >25

<table>
<thead>
<tr>
<th>TABLE 2. Univariate Analysis of Possible Risk Factors for Restenosis</th>
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<tbody>
<tr>
<td>Restenosis Group</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>No. of subjects</td>
</tr>
<tr>
<td>Age, y, mean (SD)</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>No. of lesions</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>Lp(a), mg/dL, median (range)</td>
</tr>
<tr>
<td>Cholesterol, mg/dL, mean (SD)</td>
</tr>
<tr>
<td>Family history of CAD, %</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
</tr>
<tr>
<td>Previous MI, %</td>
</tr>
<tr>
<td>Smoking after PTCA, %</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; MI, myocardial infarction.
TABLE 3. Multiple Logistic Regression Analysis of Risk Factors for Restenosis

<table>
<thead>
<tr>
<th>Coefficient Estimate</th>
<th>SE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.017</td>
<td>0.019</td>
</tr>
<tr>
<td>Sex</td>
<td>0.012</td>
<td>0.025</td>
</tr>
<tr>
<td>No. of lesions</td>
<td>0.708</td>
<td>0.337</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>0.00002</td>
<td>0.0003</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>Family history</td>
<td>0.739</td>
<td>0.518</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.08</td>
<td>0.596</td>
</tr>
</tbody>
</table>

No. of lesions mg/dL had restenosis versus 30 of 89 subjects with an Lp(a) <25 mg/dL (relative risk, 1.26; $\chi^2=1.31$; NS). A clinical history of diabetes mellitus showed borderline significance ($P=0.055$) by univariate analysis (Table 2). The number of lesions dilated and a lack of family history of coronary disease were significantly associated with restenosis ($P=0.002$ and 0.008, respectively). By multiple logistic regression analysis, only the number of lesions dilated was associated with restenosis ($P=0.03$), whereas a history of diabetes mellitus showed a trend toward significance ($P=0.07$) (Table 3).

We also analyzed the data for subjects who had only 1 vessel treated at 1 or more sites (n=131) by univariate analysis for differences in Lp(a), cholesterol levels, and target vessel classification. These 131 subjects did not differ significantly in clinical characteristics from the entire group of 126 subjects. There was no significant difference in lesion classification between the restenosis and no-restenosis groups ($P=0.2$), with 156% type A, 67% type B, and 18% type C in the restenosis group versus 25% type A, 67% type B, and 8% type C in the no-restenosis group. There was no significant difference in the target vessel of the restenosis versus the no-restenosis group ($P=0.99$), with 43% left anterior descending artery, 13% left circumflex artery, 37% right coronary artery, and 7% other sites (left main or grafts) in the recurrence group versus 44%, 13%, 36%, and 7%, respectively, in the no-restenosis group (Table 4). We also evaluated the 96 subjects who had a single lesion dilated for differences in angiographic characteristics between the restenosis and no-restenosis groups. These 96 subjects did not differ significantly in clinical characteristics from the entire group of 126 subjects. There was no significant difference in age, sex, Lp(a) level, cholesterol level, lesion classification, or target vessel between the restenosis (n=33) and the no-restenosis group (n=63).

Discussion

Whether the Lp(a) level is a significant risk factor for restenosis after PTCA remains controversial. Most of the previously published studies addressing the issue had methodological defects or constraints limiting the usefulness of the results. Various limitations of these studies included retrospective analysis, small sample size, inclusion of patients and measurement of Lp(a) within 4 weeks of acute myocardial infarction, which increases Lp(a) for at least 4 weeks; blood sampling after PTCA, the interventional nature of the study; or assay of stored samples, which can yield artifically low levels of Lp(a).

Our study is entirely prospective and observational, with a sample size estimated to detect a >1.6-fold relative risk of restenosis in patients with elevated Lp(a); potential subjects with myocardial infarction within 6 weeks were not enrolled, and all blood samples were obtained before PTCA and assayed fresh for Lp(a). When the Lp(a) level was analyzed as a continuous variable by the Mann-Whitney $U$ test, the difference in median Lp(a) level between the restenosis and no-restenosis groups was not significant. When analyzed as a categorical variable, the relative risk of restenosis for Lp(a) levels >25 mg/dL versus <25 mg/dL was 1.26, which was not statistically significant. Because our study was not designed to prove the significance of a relative risk <1.6, our data cannot exclude the possibility of a small increase in relative risk on the order of 1.1 to 1.5. The only study of comparable size to ours that showed a significant relation of Lp(a) level to risk of restenosis measured the Lp(a) level 4 weeks after angioplasty in 93 subjects who had a history of myocardial infarction within 1 month of PTCA from a total cohort of 240 subjects. These Lp(a) measurements after PTCA may have confounded the analysis of Lp(a) as a predictor of risk for restenosis. Another study of comparable size, which has been reported in abstract form, found no significant correlation of Lp(a) with restenosis but was designed to test the effect of lipid lowering on restenosis.

We analyzed several clinical and procedural characteristics for possible association with restenosis. A history of diabetes mellitus approached statistical significance by univariate and multiple logistic regression analysis. Other studies have found diabetes mellitus to be a risk factor for restenosis, whereas some have not. Violari et al reported no association between total cholesterol and restenosis in a
prospective study involving quantitative angiographic analysis of 3336 lesions, in agreement with our data and those of other investigators.9,12–14,25 Further indirect evidence that cholesterol levels are not predictive is the finding that a lack of family history of premature coronary artery disease was predictive of restenosis ($P=0.008$); such a family history would be likely in patients with elevated serum cholesterol because of familial hypercholesterolemia. We also analyzed the effect of procedural variables. Other investigators have reported that the risk of restenosis at least 1 lesion increases with each lesion dilated $15–17$ in both multivessel and multilesion angioplasty and that patients with multilesion angioplasty may have higher restenosis rates than those undergoing multivessel angioplasty.25 Roubin et al$29$ have also reported that the risk of restenosis at 1 site may be related to the number of sites dilated. In our study, the number of lesions treated was the only procedural characteristic predictive of restenosis by univariate ($P=0.002$) and multivariate ($P=0.03$) analyses.

Previous studies$6–8,19,30$ have suggested that Lp(a) may beatherogenic through its effect on the thrombotic or fibrinolytic system. The secondary aim of this study addressed this possible mechanism by measuring TAT to test thrombotic activity and APP and PAI-1 assays to measure fibrinolytic activity. We evaluated the data for any correlation between Lp(a) and TAT, APP, or PAI-1. In addition, we measured the correlation of Lp(a) levels with the ratio of thrombotic versus fibrinolytic activity (TAT-APP ratio). There was no statistically significant correlation of Lp(a) with either TAT, APP, or PAI-1, or the TAT-APP ratio. Therefore, we were not able to demonstrate that Lp(a) has a direct influence on the prothrombotic and fibrinolytic pathways. We found no significant differences between the median levels of TAT, APP, and PAI-1 in the restenosis versus no-restenosis group. The TAT-APP ratio was 2-fold higher in the restenosis group and this difference approached statistical significance ($P=0.07$), but normal subjects had a median value intermediate between the 2 patient groups, suggesting the need for caution in interpreting these results.

An elegant study in transgenic mice found evidence for in vivo suppression of plasminogen activator–mediated thrombolysis by Lp(a).$30$ It has been difficult to find such direct evidence in human subjects with elevated Lp(a) levels, although the association of elevated Lp(a) with both thrombotic disorders and defective fibrinolysis is suggested by much indirect clinical evidence and by considerable basic research. In particular, homocysteine has been shown to increase the binding of Lp(a) to fibrin.$31$ Lp(a) also binds to glycoprotein IIb$32$ and inhibits collagen-induced platelet aggregation.$33$ Because neutrophil defensins increase the binding of Lp(a) to vascular endothelial cells and to smooth muscle cells, activated neutrophils, which release defensin, may modulate the effect of Lp(a) on the vessel wall and the risk of restenosis.$34$ Direct evidence of the effect of Lp(a) on thrombosis, fibrinolysis, or the risk of restenosis is more difficult to obtain in human subjects than in animal models because methodological and ethical considerations limit the scope of direct human experimentation. Furthermore, no effective therapy is currently available to significantly lower the Lp(a) level without altering LDL as well. The possible role of inhibition of fibrinolysis by Lp(a) in the development of restenosis and other vascular diseases remains an attractive hypothesis that deserves further study.

**Acknowledgments**

This study was supported by Vascular Diseases Academic Award HL-02821 from the National Institutes of Health, Bethesda, Md (to M.B.H.). We thank Helen Gies, Kathy Tinaro, and Shirley Murray for outstanding secretarial assistance.

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doi: 10.1161/01.ATV.18.8.1281
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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