Lipoprotein (a) and anticardiolipin antibodies are risk factors for clinically relevant restenosis after elective balloon percutaneous transluminal coronary angioplasty

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Received 3 August 1999; received in revised form 24 January 2000; accepted 18 February 2000

Abstract

Recent reports have shown the importance of new risk factors for cardiovascular disease. We investigated the relationship between Lp(a), fibrinolytic parameters and anticardiolipin antibodies (aCL) and the occurrence of clinical recurrence owing to restenosis after elective balloon percutaneous transluminal coronary angioplasty (PTCA) without stenting. In 167 patients, undergoing PTCA, Lp(a) plasma levels, aCL, euglobulin lysis time (ELT), plasminogen activator inhibitor-1 (PAI-1) activity and tissue-type plasminogen activator (t-PA) plasma levels were evaluated before the procedure. During follow-up 29 patients underwent clinical recurrence due to restenosis. Lp(a) levels were significantly higher in patients with restenosis in comparison to those without ($P < 0.05$); an earlier restenosis was observed in patients with Lp(a) values $> 450$ mg/L. Kaplan–Meier survival estimate showed an earlier occurrence of restenosis in patients with base-line Lp(a) $> 300$ mg/l associated with aCL positivity. High Lp(a) plasma levels play a role in the occurrence of clinical recurrence due to restenosis after elective balloon PTCA without stenting; the association with aCL accelerates the development of restenosis. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Lp(a); Anticardiolipin antibodies; Fibrinolysis; PTCA; Restenosis

1. Introduction

High levels of lipoprotein (a) (Lp(a)) have been found to predict myocardial infarction, coronary artery disease and vein graft stenosis after bypass procedures [1–3]. Lp(a) is structurally related to important proteins involved in fibrinolysis, coagulation and cellular mitogenesis [4,5]. These observations suggest a relevant role of Lp(a) in both atherogenesis and thrombogenesis. Less clear is the role of Lp(a) in restenosis after percutaneous transluminal coronary angioplasty (PTCA). Whereas some studies suggested that Lp(a) levels are an independent predictor of restenosis [6–10], others reported that restenosis is unrelated to Lp(a) levels [11–16]. However, as recently pointed out [16], most of those studies either were partly or completely retrospective, based on assays of stored blood samples, or included patients with recent myocardial infarction, all factors capable of confounding data interpretation. Controversial results have also been reported [6–8,11,13,16,17] on the association between lipid parameters (total cholesterol, triglycerides, HDL, apolipoproteins, etc.) and restenosis after PTCA.

Recent reports have shown that antiphospholipid antibodies are a risk factor for cardiovascular disease [18,19] and that the thrombotic risk is higher when elevated Lp(a) levels coexist with antiphospholipid antibodies positivity [20]. The present prospective study was designed to evaluate the relationship between Lp(a), fibrinolytic parameters and anticardiolipin antibodies (aCL) and the occurrence of clinically relevant restenosis after elective balloon PTCA without stenting.

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2. Methods

2.1. Patients

Three hundred and fifty nine consecutive patients referred to our Department for elective PTCA were considered after having obtained their informed consent. Six patients were excluded for at least one of the following exclusion criteria: enzymatic or ECG evidence of acute myocardial infarction (AMI), clinical evidence of recent MI (less than 6 months), unstable angina class III (according to Braunwald) [21], surgical or invasive procedures in the month preceding the study, neoplastic disease, malar rash, discoid rash, oral or pharyngeal ulceration, frank arthritis; pleuritis in the absence of pulmonary embolism or left-side heart failure, pericarditis in the absence of myocardial infarction or uremia; persistent proteinuria greater than 0.5 g per day, due to biopsy-proven immune complex related glomerulonephritis, lymphopenia less than 1000/ml, antibodies to native DNA, anti-extractable nuclear antigen antibodies, antinuclear antibodies of more than 1:320, treatment with drugs known to induce aCL. One hundred seventy nine patients who underwent stent placement in addition to PTCA were excluded. Seven patients (5 males and 2 females), who underwent reocclusion of the angioplastied vessel during the first week, were also excluded from the study. Among the 167 patients included in the analysis, 43 patients were affected by stable effort angina, 77 by unstable angina, class II B according to Braunwald (they had been previously hospitalised for unstable angina in intensive care unit and then discharged), 47 had no symptoms but had a stress test positive for ischemia. The characteristics of the patients investigated are shown in Table 1.

Table 1
Characteristics of study population (n = 167)*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>130/37</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64 (34–81)</td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>96</td>
</tr>
<tr>
<td>Body mass index</td>
<td>25.8 (15.7–39.5)</td>
</tr>
<tr>
<td>Smokers</td>
<td>39</td>
</tr>
<tr>
<td>Ex smokers</td>
<td>74</td>
</tr>
<tr>
<td>Hypertension</td>
<td>70</td>
</tr>
<tr>
<td>Mean systolic blood pressure (mmHg)</td>
<td>145 (110–240)</td>
</tr>
<tr>
<td>Mean diastolic blood pressure (mmHg)</td>
<td>80 (55–120)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>25</td>
</tr>
<tr>
<td>Previous AMI</td>
<td>77</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>Unstable angina</td>
<td>77</td>
</tr>
<tr>
<td>Stable angina</td>
<td>43</td>
</tr>
<tr>
<td>Positive stress test for ischemia</td>
<td>47</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>221 (126–500)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>45 (25–79)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>180 (56–666)</td>
</tr>
</tbody>
</table>

* AMI, acute myocardial infarction; CAD, coronary artery disease.

Patients who had given up smoking since less than six months prior to the study were considered among smokers.

2.2. Angioplasty procedure

PTCA was performed through the femoral approach with steerable balloon catheter using the Judkins technique [22]. All the patients were on chronic oral treatment with aspirin (ASA, 325 mg/day) in addition to antianginal standard medications (nitrates and/or calcium blockers and/or beta-blockers). During the procedure all patients received heparin (10 000–15 000 IU i.v.), ASA 500 mg i.v. and an i.v. infusion of nitro-glycerine (5 μg/min) plus an intracoronary bolus of nitro-glycerine (250 μg) or isosorbide dinitrate (200 μg). Post-PTCA drug regimen included heparin infusion (1000–1200 IU/h) for 12–18 h, followed by subcutaneous administration (12 500 IU/day) for a week, oral treatment with ASA (325 mg/day) and antianginal therapy.

Angiograms were analysed with a quantitative visual assessment according to a modification of Brown–Dodge method [23] as previously reported [24]. The variability of measurement reproducibility of this method is 6.7% [24]. All angiograms were reviewed by three experienced angiographers who were not involved in the performance of the procedures and were blinded to the laboratory results and to the clinical outcome. With regard to coronary disease before PTCA, 70 patients had one-vessel disease, 76 had two-vessel disease and 21 had three-vessel disease. The median and range values of the diameter stenosis of the treated coronary vessels before PTCA were 90% and 75–100%, respectively. After PTCA these values were 15% and 0–40%, respectively. Successful angioplasty was defined as the restoration of normal flow with less than 50% residual stenosis without major complications.

2.3. Blood Sampling

Venous blood samples were collected by venipuncture technique using a 19 G butterfly, 1 h before PTCA in the morning (8–10:00 a.m.). Blood was drawn directly into plastic tubes containing sodium citrate 0.129 M (1/10, v/v) for the determination of Lp(a) plasma levels, euglobulin lysis time (ELT), plasminogen activator inhibitor-1 (PAI-1) activity and tissue-type plasminogen activator (t-PA) concentration. Plasma samples obtained after centrifugation were stored at −80°C except for Lp(a) and ELT determinations, which were performed on fresh samples. Sera for testing aCL were obtained by centrifugation of blood collected without anticoagulant at 1300 × g for 10 min and stored at −20°C. Stored plasma and serum were assayed within 15 days for the determination of all parameters.
Table 2
Base-line Lp(a) and haemostatic factors

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>No restenosis related recurrence</th>
<th>Restenosis related recurrence</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a) (mg/l)</td>
<td>126 (10–1260)</td>
<td>121 (10–1200)</td>
<td>193 (10–1174)</td>
<td>0.05</td>
</tr>
<tr>
<td>ELT (h)</td>
<td>6 (2–10.5)</td>
<td>6 (2–10.5)</td>
<td>6.5 (2-10.5)</td>
<td>ns</td>
</tr>
<tr>
<td>t-PA (ng/mL)</td>
<td>12.1 (3–27.9)</td>
<td>12.4 (3–27.2)</td>
<td>12 (4–27.9)</td>
<td>ns</td>
</tr>
<tr>
<td>PAI-1 (IU/mL)</td>
<td>7.4 (3–38.1)</td>
<td>7.4 (3–38.1)</td>
<td>7.6 (3–25)</td>
<td>ns</td>
</tr>
</tbody>
</table>

* ELT, euglobulin lysis time; Lp(a), lipoprotein (a); PAI-1, plasminogen activator inhibitor-1 activity; t-PA, tissue-type plasminogen activator antigen.
* comparison between patients with and without restenosis related recurrence.

2.4. Laboratory test

Lp(a) was assayed by ELISA (TintElyze Lp(a), Biopool, Umea, Sweden); (90th percentile of our control population = 450 mg/l). PAI-1 activity was evaluated according to Chmielewska [25] by a chromogenic method (Spectrolyse (fibrin), Biopool) (control range 2–15 IU/ml). The t-PA antigen plasma concentration (control range 1.7–10 ng/ml) was assayed by ELISA (TintElyze t-PA, Bio-Pool). Euglobulin lysis time (control range 2.5–5 h) was determined according to Chakrabarti [26]. The aCL assay was performed by ELISA (First Cardiolipin, Europital, Trieste, Italy) and aCL levels were reported in GPL units (for IgG) and in MPL units (for IgM). On the basis of analysis of several hundreds of normal sera performed in our laboratory in the past, and according to the literature, values above 20 IU for either IgG or IgM were considered abnormal [27,28].

2.5. Follow-up

Patients were clinically followed up for a mean time of 20 months. During follow-up they were invited to contact the cardiologists when symptoms referable to cardiac ischemia occurred and to refer to the hospital for any subsequent clinical evaluation. Ergometric tests were performed 1, 3 and 6 months after PTCA. During the follow-up period, for ethical reasons, angiography was again performed only in patients with clinical recurrence defined as either recurrent anginal attacks, a positive treadmill test (Bruce protocol) [29], or a positive stress test with 201Tl-scintigraphy. Restenosis was defined by the presence of a decrease > 50% of gain in luminal diameter achieved by PTCA [30].

2.6. Statistical analysis

The results are expressed as median and range because of their skewed distribution. Preliminary statistical analysis was performed using the Wilcoxon’s signed rank test, Fisher’s exact test or Kruskal–Wallis test. Correlation coefficients were calculated with Spearman’s rank test. Multivariate analysis was assayed by logistic or linear regression. Survival estimate was obtained by Kaplan–Meier test. P values < 0.05 were considered significant.

3. Results

All the 167 patients showed up for the non-invasive follow-up. Thirty-three patients underwent angiography for clinical recurrence and in 29/167 (17%) restenosis of the angioplastied artery was demonstrated. The mean time of restenosis was 5 ± 3 months.

3.1. Lp(a), fibrinolysis and a CL

The results of base-line Lp(a) and haemostatic parameters are reported in Table 2. Base-line Lp(a) levels were > 300 mg/l in 55/167 patients. Lp(a) levels were negatively correlated with PAI-1 activity (r = − 0.22, P < 0.01) but this correlation was no longer present after adjustment for the presence of aCL, diabetes mellitus and smoking habits, or the levels of total cholesterol and triglycerides. No correlation was observed between Lp(a) and the other haemostatic factors. However, Lp(a) plasma levels > 300 mg/l showed a trend to be associated with prolonged ELT (≥ 5 h, P = 0.06). Eighteen patients had positivity for aCL and six of them showed Lp(a) > 300 mg/l. No significant correlation was found between aCL positivity and Lp(a) levels.

3.2. Lp(a), clinical and angiographic factors

No correlation was found between Lp(a) levels and angiographic characteristics such as stenosis before PTCA and the number of diseased vessels as well as the other clinical characteristics except for smoking habitus which was associated with lower Lp(a) levels (P < 0.01). The entire population was divided into two subgroups in relation to Lp(a) levels: above and below 300 mg/l. No significant differences in clinical and angiographic parameters were found between patients of the two subgroups.
3.3. Restenosis-related clinical recurrence

Base-line Lp(a) median levels were significantly higher in patients with recurrence and restenosis in comparison to those without (respectively, 193 vs. 121 mg/l, \(P < 0.05\); see Table 2). A multivariate logistic regression showed an association of diabetes mellitus with restenosis (8 of the 25 diabetic patients underwent restenosis) and confirmed that Lp(a) is an independent predictor of restenosis-related clinical recurrence, after adjustments for the presence of aCL, total cholesterol, triglycerides, diabetes mellitus, smoking habitus.

Kaplan–Meier survival estimate showed an earlier occurrence of restenosis in patients with Lp(a) > 450 mg/l (Fig. 1, \(P < 0.05\)) as well as in patients with Lp(a) levels > 300 mg/l if associated with aCL positivity (Fig. 2, \(P < 0.05\)). No relationship was found either between levels of total cholesterol, HDL cholesterol, triglycerides, t-PA, PAI-1, ELT and restenosis, or between aCL positivity and restenosis.

4. Discussion

In this study we report that high Lp(a) levels represent a risk factor for restenosis-related clinical recurrence after elective balloon PTCA and that the contemporary presence of aCL increases this risk in patients with Lp(a) > 300 mg/l. Our study is hardly comparable with most of previous investigations on the role of Lp(a) levels in angiographic restenosis. Those studies have various limitations including retrospective analysis, small sample size, inclusion of patients within 4 weeks of acute myocardial infarction, blood sampling after PTCA, or assay of stored samples which can yield artificially low levels of Lp(a) [8,11–13,17,31,32].

4.1. Role of Lp(a) and lipids in restenosis-related clinical recurrence after PTCA

We found no significant relationship between either cholesterol or triglycerides levels and clinical recurrence after PTCA. Contradictory results have been reported over the last years on this issue and our data confirm the absence of consistent data to support a role of lipid levels as risk factor for restenosis.

The correlation between restenosis and Lp(a) levels observed by us is in agreement with data found by Desmarais et al. [17]. However, they measured the Lp(a) levels 4 weeks after PTCA in patients who had a history of myocardial infarction within 1 month of PTCA and these Lp(a) measurements after PTCA may have confounded the analysis. In a recent well-designed investigation, Alaigh et al. did not find any difference in Lp(a) levels between restenosis and no restenosis patients after PTCA [16]. However, the end-point of our study was clinical recurrence due to restenosis and not restenosis per se so that the two studies are not comparable. Plasma Lp(a) has recently been demonstrated to be not a predictor for restenosis after elective high-pressure coronary stenting [33]. However, due to differences in the pathogenesis of restenosis after coronary stenting and after balloon PTCA, the two studies are not comparable.

It has been reported that patients with unstable angina and recent myocardial infarction have a transient increase in the plasma levels of Lp(a) during acute phases of disease [34]. We did not find any significant difference in Lp(a) levels between patients with unstable and those with stable angina, whereas patients with recent infarction were excluded from our study. By univariate, but not by multivariate analysis we found an inverse correlation between PAI-1 activity and Lp(a). This relationship had been already reported in healthy controls [35], but had not been confirmed in other studies [36].

Different mechanisms may link high Lp(a) levels to restenosis after PTCA. Lp(a), in addition to inhibiting
plasminogen binding to the surface of endothelial cells and reducing the activity of t-PA [4], is able to promote human smooth muscle cells proliferation by interfering with transforming growth factor activation [5].

4.2. Co-operation of the presence of aCL and high Lp(a) levels in restenosis-related clinical recurrence after PTCA

In this study no relation was observed between the presence of aCL and clinical restenosis but we demonstrated that the contemporary presence of aCL and elevated Lp(a) levels markedly increased the risk of restenosis-related clinical recurrence after PTCA. Antiphospholipid antibodies have been found to be associated with arterial and vein thrombosis [37,38]. However, conflicting results have been reported on the role of aCL in coronary artery disease [39,40] and the mechanism by which aCL arise and their relationship to the thrombotic events have not been clearly elucidated [38].

A higher risk for arterial thrombosis in aCL positive patients with high Lp(a) levels has been reported in clinical conditions such as Systemic lupus eritematosus [20] and Rheumatoid Arthritis [41]. Recently, increased levels of Lp(a) have been reported to be associated with a low fibrinolytic activity in patients with the antiphospholipid syndrome [42]. In our patients we could not observe a clear-cut relationship among Lp(a) levels, aCL and the fibrinolytic parameters. However, a reduced fibrinolytic activity is just one of the possible mechanisms linking the presence of both aCL and elevated Lp(a) levels to an increased risk of clinical recurrence after PTCA. Lp(a) has been found to be related to the acute phase reaction. Thus, the increased risk for restenosis in patients with high Lp(a) levels and aCL could suggest a role for an inflammatory condition predisposing to an enhanced reaction to the balloon injury.

4.3. Limitations of the study

One limitation of this study is the fact that we did not determine Lp(a) polymorphisms. Apo(a) shows a size polymorphism, with individual isoforms ranging in apparent molecular weights from about 250–800 KD. Small apo(a) has a significant association with coronary artery disease and the combination of high Lp(a) levels and small size apo(a) isoform has been reported to be the greatest risk factor for coronary artery disease in hypercholesterolemic patients [43] and to be associated with very low fibrinolytic activity [44]. So we cannot exclude that this combination could be a more relevant risk factor for restenosis after PTCA than Lp(a) levels alone. Another limitation may be the absence of systematic follow-up angiography. As specified in Section 2, in the present study angiography has been performed only in patients with clinical recurrence or inducible ischemia. Thus, we cannot draw any conclusion on the effect of Lp(a) and aCL on restenosis ‘per se’. However, we chose a clinically relevant definition of recurrence (that required recurrence symptoms or positive stress test) with angiographically demonstrated restenosis. This definition seems appropriate because borderline luminal diameter narrowing scarcely correlates to clinical symptoms and prognosis [45]. Our policy, strictly limiting invasive procedures, is in agreement with recent ACC/AHA guidelines for coronary angiography [46]. Finally, it should be remembered that the results of this study are relevant only for patients undergoing elective balloon PTCA without stent implantation.

5. Conclusion

This study shows for the first time that aCL positivity and high Lp(a) levels act synergistically in increasing the risk of clinically relevant restenosis after elective balloon PTCA. These results are relevant because, although stenting is largely used, a significant proportion of patients have still indication to PTCA without stenting. At present, Lp(a) plasma levels are not modifiable by drugs commonly used in clinical practice, but the effects of new agents able to lower Lp(a) concentration or apheresis could be interestingly explored in this high risk clinical condition [9]. Moreover, symptomatic patients with antiphospholipid antibodies have been demonstrated to take advantage from oral anticoagulant therapy [47–50]. These results suggest an opportunity to design studies aimed at elucidating the effect of more aggressive therapeutic strategies in selected groups of patients in whom the risk of restenosis related recurrence is particularly high in relation to the contemporary presence of high Lp(a) plasma levels and aCL positivity.

References


