



Lipoprotein (a) in young individuals as a marker of the presence of ischemic heart disease and the severity of coronary lesions

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The purpose of this study was to evaluate whether high levels and small isoforms of lipoprotein (a) [Lp(a)] are markers of risk of early myocardial infarction and markers of the severity of coronary atherosclerosis. Lp(a) levels and small apo(a) isoforms were higher in 222 patients than in 199 controls ($p < 0.001$). In patients, Lp(a) ≥ 30 mg/dL was associated with the presence of coronary lesions ($p = 0.007$) and the severity of coronary atherosclerosis ($p = 0.002$). The present study suggests that Lp(a) levels and small isoforms are markers of early myocardial infarction and that Lp(a) levels ≥ 30 mg/dL are associated with severe patterns of coronary atherosclerosis.

Key words: lipoprotein (a), ischemic heart disease, angiography, fibrinolysis, atherothrombosis.

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Besides cardiovascular risk factors, hemostatic balance may play a role in the onset and progression of atherothrombosis. Lipoprotein (a) [Lp(a)], a part of low density lipoprotein cholesterol, can exert a pro-atherogenic and hypofibrinolytic effect. The size of its glycoprotein component, apolipoprotein(a) [apo(a)], varies significantly¹ as a result of which different isoforms have been identified. Lp(a) concentration is highly inheritable and there is no compelling evidence that Lp(a) levels can be lowered by pharmacological interventions other than hormone replacement therapy.³ Although there is evidence indicating that the risk of ischemic heart disease increases with high Lp(a) levels,⁴⁻⁶ and/or small apo(a) isoforms,⁷ the specific mechanisms involved are unknown. Basically, Lp(a) can promote atherothrombosis either by decreasing fibrinolytic activity^{7,8} or by enhancing the growth of atheromatous lesions.⁹ The first effect can be explained by two different mechanisms: the competition with plasminogen to bind fibrin¹⁰ and up-regulation of the expression of plasminogen activator inhibitor-1 (PAI-1).¹¹ The PAI-1 promoter 4G/5G polymorphism modulates PAI-1 levels, but whether this polymorphism influences the effect of Lp(a) on PAI-1 levels has not yet been investigated.

The relationship between Lp(a) levels and the severity of coronary atherosclerosis in patients with unstable angina or acute myocardial infarction (MI) has been analyzed in several studies with controversial results,¹²⁻¹⁴ while the potential value of small apo(a) isoforms in predicting severe angiographically demonstrable atherosclerosis

remain unclear. In this study we simultaneously analyzed Lp(a) levels, apo(a) isoforms and the angiographic pattern (presence/absence of coronary lesions and severity of the coronary atherosclerosis) in young stable survivors of MI. We also determined PAI-1 levels and other fibrinolytic parameters to provide a preliminary insight into the mechanism by which Lp(a) may promote atherothrombosis. These variables were also analyzed in controls in order to assess their potential role as markers of ischemic heart disease in young people.

Design and Methods

Our study included 222 subjects, aged < 51 years who had had a MI¹⁵ (Table 1). None of them had a previous history of stroke or malignancy. Blood sampling was performed in a clinically stable condition ≥ 3 months after the acute coronary event. Coronary angiograms were analyzed to establish the absence or presence (luminal narrowing $\geq 70\%$) of significant coronary lesions and the severity of coronary atherosclerosis, which was determined using a stenosis score.¹⁶ According to this score, coronary atherosclerosis was graded as absent (0 points), mild (1-9 points), or severe (≥ 10 points). One hundred and ninety-nine unrelated Caucasian controls from the same geographical area as the patients and with a similar age and sex distribution were enrolled in the study (Table 1). All controls were apparently healthy and none of them had a personal history of thrombotic disease or malignancy. Classical cardiovascular risk factors were

Table 1. Basic characteristics of the patients and controls.

	Patients (n=222)	Controls (n=199)	p	
Age at sampling (years)	44.9±5.1	43.8±8.3	0.103	
Sex (male/female)	198/24	179/20	0.799	
Total cholesterol (mmol/L)	4.92±1.00	5.55±1.00	<0.001	
HDL-cholesterol (mmol/L)	1.11±0.25	1.22±0.32	0.002	
LDL-cholesterol (mmol/L)	3.04±0.84	3.63±0.94	<0.001	
VLDL-cholesterol (mmol/L)	0.67 (0.39-1.27)	0.62 (0.31-1.27)	0.032	
Triglycerides (mmol/L)	1.49 (0.84-2.85)	1.21 (0.65-2.60)	<0.001	
	Patients (n=222)	Controls (n=199)	OR (95% CI)	
			Crude	Adjusted
Smokers (%)	89.2	41.7	11.53 (6.93-19.18)	12.68 (6.52-24.66)
Systemic hypertension (%)	36.0	11.1	4.53 (2.69-7.63)	5.45 (2.27-13.06)
Diabetes mellitus (%)	18.9	9.5	2.21 (1.34-3.95)	4.96 (1.58-15.57)
Dyslipidemia (%)	67.1	27.6	5.34 (3.52-8.12)	3.75 (2.11-6.65)
Obesity (%)	26.6	13.1	2.41 (1.45-4.01)	2.80 (1.21-6.48)

Continuous variables were compared using the Student's t-test (normal distribution) or the Mann Whitney U-test (without normal distribution). Crude OR were obtained from the χ^2 test and adjusted OR from binary logistic regression analyses including in the model smoking, systemic hypertension, diabetes mellitus, dyslipidemia and obesity. Systemic hypertension was defined as a previous history, the presence of a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg, and/or the use of anti-hypertensive drugs; diabetes mellitus as a previous history, the presence of a fasting glucose > 126 mg/dL, and/or the use of antidiabetic drugs; dyslipidemia as a previous history, the presence of a total cholesterol level > 250 mg/dL and/or triglyceride level > 175 mg/dL, and/or the use of statins and/or fibrates; smokers as current or previous smokers in the last 10 years, and finally, obesity was defined as a body mass index > 30 kg/m². HDL: high density cholesterol; LDL: low density cholesterol; VLDL: very low density cholesterol; OR: odds ratio; CI: confidence interval.

defined as a previous history or the presence at the time of the study or the use of drugs for systemic hypertension, diabetes mellitus, dyslipidemia or smoking. The study was approved by the local Ethics Committee. All patients and controls were informed about the study and gave their written consent to participation in agreement with the Declaration of Helsinki.

Blood samples were anticoagulated (0.13 mmol/L trisodium citrate), centrifuged (1,500xg, 30 min, 4°C), and frozen in aliquots (-80°C). Samples were collected into EDTA for DNA extraction and serum was used to determine glycemia and the lipid profile. Plasma Lp(a) levels were quantified using an ELISA kit [Macra Lp(a), Terumo]. Apo(a) phenotypes were determined as reported by Kamboh *et al.*,¹⁷ with slight modifications.² Based on their major apo(a) isoform, subjects were classified as having small (B, S1 and S2) or large apo(a) (S3, S4 and S5) isoforms. PAI-1 and t-PA antigen levels were determined with ELISA kits (Tint Elize PAI-1 and Imulyse t-PA, Biopool) and PAI-1 activity levels and euglobulin lysis time were assayed as previously described.² Fibrinogen levels were determined by a stan-

Table 2. Lipoprotein (a) levels, apo(a) isoforms, inflammatory and fibrinolytic variables in patients and in controls.

	Patients (n=222)	Controls (n=199)	p	
Lp(a) (mg/dL)	13.15 (1.00- 48.70)	6.00 (0.40-26.54)	<0.001	
hs-CRP (mg/L)	1.55 (0.47-6.28)	1.06 (0.34-3.68)	0.002	
Euglobulin lysis time (min)	228.7±74.4	177.8±61.7	<0.001	
t-PA Ag (ng/mL)	10.3±5.7	11.2±3.9	0.208	
Fibrinogen (g/L)	2.87±0.71	2.49±0.48	<0.001	
PAI-1 Ag (ng/mL)	26.2 (13.4-56.0)	19.0 (7.0-37.3)	<0.001	
PAI-1 Act (U/mL)	20.2 (4.0-34.0)	12.4 (0.0-32.7)	<0.001	
	Patients (n=222)	Controls (n=199)	OR (95% CI)	
			Crude	Adjusted
Lp(a) levels > 30 mg/dL (%)	27.0	7.5	4.54 (2.48-8.31)	3.02 (1.28-7.09)
Small apo(a) as the major isoform (%)	30.6	15.6	2.39 (1.48-3.86)	1.78 (0.89-3.57)

Continuous variables were compared with the Student t-test (normal distribution) or the Mann Whitney U-test (without normal distribution). Crude OR were obtained from the χ^2 test and adjusted OR from binary logistic regression analyses including in the model smoking, systemic hypertension, diabetes mellitus, dyslipidemia and obesity. PAI-1 Ag: plasminogen activator inhibitor-1 antigen; PAI-1 Act: PAI-1 activity; t-PA Ag: tissue type plasminogen activator antigen; hs-CRP: high sensitivity C-reactive protein; OR: odds ratio; CI: confidence interval.

dard coagulation method.¹⁸ The PAI-1 promoter 4G/5G polymorphism was analyzed with an allele-specific polymerase chain reaction technique.¹⁹ The presence of factor V Leiden and prothrombin 20210A variant was assessed with commercial kits (Roche Molecular Biochemicals). High sensitivity C reactive protein (hs-CRP) levels were measured by nephelometry (Behring Nephelometer, Behringwerke AG). Blood glucose, total cholesterol and triglyceride levels were determined with an autoanalyzer (DAX Technicon) and LDL-cholesterol calculated using Friedewald's formula.

In accordance with the one-sample Kolmogorov-Smirnov test, continuous variables with a normal distribution were summarized as a mean±SD and those without a normal distribution as a median (percentiles 10%-90%); means were compared with the unpaired Student's t-test or the Mann Whitney U-test, respectively. The χ^2 test was performed to compare categorical variables and gave odds ratios (OR) with 95% confidence intervals (CI). Adjusted ORs and p values were calculated from binary logistic regression models, including those cardiovascular risk factors statistically associated with the dependent variable in the univariate analysis. Spearman's correlation coefficients were applied where necessary. Values of $p < 0.05$ were considered to be statistically significant. All data were analyzed with SPSS Release software, 11.5 version for Windows (SPSS Inc).

Table 3. Lp(a) and angiographic findings in patients who underwent coronary angiography (n=180).

	Significant lesions				Severity of coronary atherosclerosis				
	Yes (n=156)	No (n=24)	Crude p	Adjusted p	Severe (n=29)	Mild (n=129)	Absent (n=22)	Crude p	Adjusted p
Lp(a) level ≥30 mg/dL (%)	30.1	4.2	0.007	0.041	48.3	25.6	4.5	0.002	0.011* 0.020 [†]
Small apo(a) isoform (%)	30.1	25.0	0.608	0.919	37.9	27.9	27.7	0.548	0.648* 0.307 [‡]

See "Design and Methods" for definition of the angiographic findings. Crude p values were obtained from the χ^2 test and adjusted p values from binary logistic regression analyses including dyslipidemia in the model. *severe versus absent. [†]severe versus mild. Lp(a): lipoprotein(a); apo(a): apolipoprotein(a).

Results and Discussion

Patients had higher VLDL-cholesterol and triglyceride levels and lower total cholesterol, HDL- and LDL-cholesterol levels than had controls (Table 1). In addition, cardiovascular risk factors and obesity were more frequent in patients than in controls (Table 1). Lp(a) levels were higher in patients than in controls ($p < 0.001$), and both Lp(a) levels ≥ 30 mg/dL and small apo(a) isoforms were more frequent in patients (Table 2). Moreover, small apo(a) isoforms were associated with higher Lp(a) levels in both groups ($p < 0.001$). In the control group, neither Lp(a) levels nor apo(a) isoforms correlated with cardiovascular risk factors or lipid profile fractions (*data not shown*). In comparison to controls, patients had impaired fibrinolytic activity, evidenced by a prolonged euglobulin lysis time, and higher levels of PAI-1 antigen and activity ($p < 0.001$). Patients also showed increased levels of inflammatory markers, such as fibrinogen and hs-CRP (Table 2). As regards the 4G allele, an unfavorable genetic profile was observed in patients (OR 1.46, 95% CI 1.22-1.74). There was a significant association between the presence of the 4G allele and higher levels of PAI-1 antigen in controls ($p = 0.010$), but not in patients ($p = 0.775$). No significant correlation was observed between PAI-1 antigen and Lp(a) levels in the total population ($p = 0.343$), in the control group ($p = 0.504$), or in the patient group ($p = 0.609$), and similar results were observed after adjustment for the 4G/5G polymorphism (*data not shown*). Differences were not observed in the prevalence of prothrombin 20210A variant or factor V Leiden (patients versus controls: 3.6% versus 4.0% $p = 0.823$ and 1.4% versus 2.0% $p = 0.598$, respectively).

Statin treatment (given to 71.2% of the patients) was not associated with significant differences in Lp(a) levels ($p = 0.083$). However, patients on aspirin (88.7%) did have lower Lp(a) levels ($p = 0.043$). Following medical criteria, 180 patients underwent coronary angiography (at a median of 4.9 months after blood sampling). Lp(a) levels ≥ 30 mg/dL were more frequent in the group with significant coronary stenoses than in those without and also significantly associated with the most severe coro-

nary atherosclerotic patterns (Table 3). Similar findings were obtained when only the 111 patients whose coronary angiograms were performed within 6 months of blood sampling were considered ($p < 0.03$). However, neither small apo(a) isoforms (Table 3) nor fibrinolytic parameters (*data not shown*) were associated with the presence of coronary lesions or with the most severe coronary atherosclerotic patterns.

The present study simultaneously assessed the usefulness of Lp(a) levels and isoforms in the evaluation of young stable MI survivors. The most relevant finding was that Lp(a) levels ≥ 30 mg/dL were not only associated with the presence of coronary stenosis but also with the severity of the coronary atherosclerosis. An increased Lp(a) level (≥ 30 mg/dL) is thought to be an important cardiovascular risk factor,⁶ especially in young and middle-aged men and women.⁴ Furthermore, both Lp(a) levels and small apo(a) isoforms have been identified as independent cardiovascular risk factors.²⁰ In line with this finding, our study showed a significantly higher percentage of these apo(a) isoforms in patients than in controls. A trend towards an association between higher Lp(a) levels and severe patterns of coronary atherosclerosis has been reported¹³ but no simultaneous assessment with apo(a) isoforms has so far been published. Our results indicate that Lp(a) levels ≥ 30 mg/dL are associated with the presence of coronary lesions and also with severe coronary atherosclerosis, even when cardiovascular risk factors and specific treatments (statins and/or aspirin) were taken into account. Although apo(a) isoforms correlated with Lp(a) levels, having predominantly small apo(a) isoforms did not predict the presence or the severity of coronary lesions. These apparently contradictory results are probably due to the fact that those individuals with *predominantly small* apo(a) isoforms can simultaneously express small and large isoforms, and therefore have intermediate Lp(a) levels.

The pathogenesis of atherothrombosis is complex and Lp(a) may explain at least part of the interaction between lipids and fibrinolysis. Increased Lp(a) levels and decreased fibrinolytic activity were both observed in our study, supporting their combined negative role in the pathogenesis of atherosclerosis.

In summary, our study shows a direct relationship between Lp(a) levels and adverse patterns of coronary atherosclerosis in young MI survivors. The present study also suggests that high Lp(a) levels and the presence of major small apo(a) isoforms may be useful markers of premature MI.

EZ designed the study, recruited patients and controls, participated in the discussion and wrote the final version of the manuscript. CF, LR, RC and AE performed all the specific techniques for the measurement of Lp(a) levels, PAI-1 antigen levels, PAI-1 activity levels, the determination of apo(a) isoforms, the determination of the PAI-1 promoter 4G/5G polymorphism, and the detection of factor V Leiden and the prothrombin 20210A variant. They all prepared the "Design and Methods" section referred to the laboratory techniques employed. AE also contributed significantly to the discussion and revised the manuscript for important intellectual content. MAA, AO and MAP gave administrative and logistic

support, reviewed the coronary angiograms, computed the stent score of each patient and participated in the statistical analysis of the results. LA contributed to reviewing the literature, collecting clinical data and participating in the statistical analysis of the results. FE contributed to the interpretation of the data and was greatly involved in the critical revision of this manuscript. The authors declare that they have no potential conflicts of interests and that they have all read and approved the final version of the manuscript.

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