DOTTORATO IN MEDICINA INTERNA CLINICO-SPERIMENTALE- CURRICULUM FISIOPATOLOGIA E CLINICA DELLE MALATTIE RENALI, CARDIOVASCOLARI E DELL'IPERTENSIONE ARTERIOSA, CICLO XXXII

"Serum lipoprotein(a) predicts acute coronary syndromes in patients with severe carotid stenosis."

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To my wife Daniela and my children Nicole, Emily and Mathys.

I thank Prof. Fabrizio Montecucco for his invaluable support.

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SUMMARY

Atherosclerotic cardiovascular disease (CVD) represents a significant health issue in the present and future worldwide populations, thus implying a crucial role in their risk assessment in primary and secondary prevention. Among the classical risk factors, lipids and lipoproteins serum concentrations are used to estimate the risk of CVD and guide therapeutic decision-making. In the present study, we focused on the prognostic value of the lipoprotein (a) [Lp(a)], a < 70 nm diameter low-density lipoprotein (LDL) that can freely flux across the endothelial vascular barrier and settle within the arterial wall [1]. Both pathophysiological and prognostic values of Lp(a) remain unclear. Given his structural similarity to plasminogen and the oxidized phospholipid load, Lp(a) shows pro-coagulant and pro-inflammatory effects [2]. Lifelong exposure to higher Lp(a) levels were strongly and causally associated with an increased risk of atherosclerotic cardiovascular disease in Mendelian randomization studies [3, 4], but observational studies show a weaker association compared with LDL concentration and unclear cut-off [5, 6]. In the present study, the prognostic value of Lp(a) and its correlation with intraplaque features were assessed in patients with severe carotid artery stenosis undergoing endarterectomy (n=180). The cut-off value of 10 mg/dL for serum Lp(a) was selected to predict 24-month follow-up acute coronary syndrome (ACS). Besides, the association between serum Lp(a) and intraplaque lipids, collagen, inflammatory and vascular cells was assessed. Serum Lp(a) levels were measured by nephelometric assay. Patients with high Lp(a) had similar comorbidities, medications and laboratory parameters as compared to low Lp(a) levels. At 24-month follow-up, patients with high

Lp(a) had more ACS as compared to low levels. Histological parameters within plaques were comparable in the study groups. No significant correlation between Lp(a) serum levels and intraplaque parameters was found, except for a weak positive association with smooth muscle cells in upstream plaque portions. When adjusted for gender, presence of dyslipidaemia and chronic coronary artery disease, Lp(a) ≥10 mg/dL remained predictive for ACS. In conclusion, Lp(a) determination could be useful to predict ACS in patients with severe carotid stenosis.

GENERALITIES

Lp (a) is a low-density lipoprotein synthesised and secreted by the liver and comprises a lipid core of LDL cholesterol and apolipoprotein B-100, linked by a single interchain disulphide bridge to a unique glycoprotein, apoprotein (a) [7, 8]. The physiological function of Lp (a) is unclear, but several observational studies, including meta-analyses and genomic investigations, suggest that high Lp (a) concentrations are associated with myocardial infarction, stroke and calcific aortic valve stenosis [9-11]. The most recent international guidelines recommend considering circulating Lp (a) measurement in people with high CVD risk (i.e. patients with ≥ 10% of 10-year risk of a fatal cardiovascular event, patients personal or a family history of premature CVD, familial hypercholesterolemia or recurrent CVD despite optimal lipid-lowering treatment) as well as in patients who have an estimated 10-year risk that is close to the threshold between high and moderate risk, in order to provide significant improved risk reclassification under certain conditions that are not reflected by the SCORE system [12]. The cut-off value of serum Lp(a) associated with increased CVD risk was suggested at above 50 mg/dL [13]. However, in a recent meta-analysis, including eleven secondary prevention studies, the prognostic cut-off value of Lp (a) in established CVD remains unclear, and no threshold risk effect was found [13]. Moreover, data in secondary prevention populations are limited to heterogeneous trials, raising questions regarding the value of Lp (a) as a clinically useful biomarker for risk assessment in this setting.

EXPERIMENTAL PART

Study aim

This study aimed to explore the predictive role of Lp (a) for the development of ACS and his relationship with atherosclerotic plaque components in a cohort of high-risk patients with advanced atherosclerosis (i.e. severe carotid artery stenosis undergoing carotid endarterectomy [CEA]).

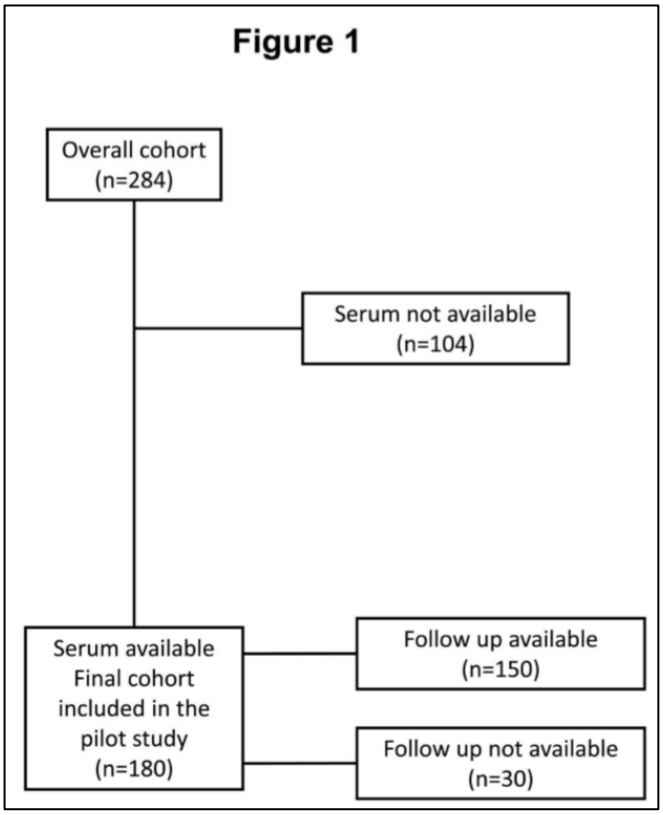
A research article from this project has been published in the *European Journal of Clinical Investigation 2018 Mar;48(3) doi: 10.1111/eci.12888).*

Materials and methods

This is a pilot sub-study of a previously published cohort of patients suffering from severe carotid artery stenosis undergoing CEA [14]. Briefly, between March 2008 and November 2012, we prospectively included 284 patients with severe extracranial high-grade internal carotid artery stenosis (defined as >70% luminal narrowing diagnosed at ultrasound Doppler), who underwent CEA at a single hospital (San Martino Hospital) in Genoa, Italy.

Among the total cohort, only 180 samples were available for measurement of serum Lp(a) levels, as reported in **Figure 1**.

All 180 patients were included in this sub-study analysis, acknowledging that 24-month follow up information was available for only 150 patients (17% of missing data at follow-up was accepted).



All patients underwent elective carotid endarterectomy according to the recommendations published by the North American Symptomatic Carotid Endarterectomy Trial (NASCET) [15], the European Carotid Surgery Trial (ECST) [16], and the Asymptomatic Carotid Surgery Trial (ACST) [17]. On total 180 patients enrolled, 136 patients were asymptomatic, while 44 were symptomatic for ischemic stroke (meaning they presented a first episode of ischemic stroke in the period between 30 and 10 days before CEA).

Ischemic stroke was defined as ipsilateral focal neurological deficit of acute onset lasting >24 hours. The day before endarterectomy, blood samples were obtained by peripheral venipuncture at fasting state to collect both serum and plasma and to perform blood parameters. Internal carotid plaques were shortly processed (within 10 minutes on ice temperature) after CEA. All

atherosclerotic plaques were cut perpendicular to the long axis through the point of maximum stenosis to obtain two portions (upstream and downstream the blood flow). All patients were prospectively followed every six months with a clinical examination up to 24 months. Medications reported in **Table 1** were not modified in the two months before enrolment. Exclusion criteria were reported in previous publications from the same cohort of patients [14,18].

Based on recent evidence, we assumed values of Lp(a) lower than 10 ng/ml as the threshold defining low risk of CVD [19, 20]. Therefore, patients were categorized low Lp(a) (<10 mg/dL, defined as controls) and high Lp(a) (≥10 mg/dL, defined as cases).

The Medical Ethics Committee of San Martino Hospital approved the study, and all participants provided written informed consent. The study was conducted in compliance with the Declaration of Helsinki.

Study endpoints

The primary end-point of the study was to determine whether high Lp(a) levels, would predict the occurrence of an ACS at 24-month follow-up after the CEA. The secondary endpoint was to determine the potential correlations between circulating Lp(a) and histological parameters of intraplaque inflammation in upstream and downstream portions of carotid plaques. Two independent investigators who were blinded to the biochemical and histological analyses adjudicated the study endpoints. Information was obtained during a check-up visit at 24 months and was further confirmed by

checking patients' medical file, mainly, targeting medical history relevant to the study endpoint.

Detection of circulating molecules

Serum C-reactive protein (CRP) levels were measured by colourimetric enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN), following the manufacturer's instructions. Lp(a) levels were determined in serum samples using a nephelometry assay (Immage Immunchemie System, Beckmann Coulter, Italy) with a polyclonal antibody directed against the apoprotein(a)-domain of Lp(a) in an assay insensitive to apoprotein(a) isoforms. The limits of detection were 15.62 pg/mL for C-reactive protein and 2 mg/dL for Lp(a). Mean intra- and interassay coefficients of variation were <8% for all markers. Glucose, triglycerides, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, fibrinogen and d-dimer were routinely measured.

Sirius Red staining for collagen content

Eight sections per each portion separated by 105 μm from each other (upstream and downstream the blood flow) were rinsed with water and incubated with 0.1% Sirius red (Sigma Chemical Co) in saturated picric acid for 90 minutes. Sections were rinsed twice with 0.01 N HCl for 1 minute and then immersed in water. After dehydration with ethanol for 30 seconds and coverslipping, the sections were photographed with identical exposure settings each section under either conventional polychromatic or polarized light microscopy. Total collagen content was evaluated under polychromatic

light (Sirius red staining). Interstitial collagen subtypes were evaluated using polarized light illumination; under this condition, thicker Type I collagen fibres appeared orange or red, whereas thinner Type III collagen fibres were yellow or green [18]. Quantifications were performed with MetaMorph™ 6 software. Data were calculated as percentages of the stained area on total lesion area.

Oil Red O staining for intraplaque lipid content

Eight sections separated by 105 μm from each other per each portion (upstream and downstream the blood flow) were incubated in 60% isopropanol for 2 minutes and then in Oil Red O solution for 20 minutes and rinsed in phosphate-buffered saline. Sections were then counterstained with Mayer's hemalune solution and rinsed in distilled water. Quantifications were performed with MetaMorph™ 6 software. Data were calculated as percentages of the stained area on total lesion area.

Immunostaining of carotid plaques

Eight sections separated by 105 μm from each other per each portion (upstream and downstream the blood flow) were fixed in acetone at room temperature and immunostained with specific antibodies anti-human smooth muscle actin (smooth muscle cells [SMC], diluted: 1:100; Dako Corporation, Glostrup, Denmark), anti-human CD66b (neutrophils, diluted: 1:50; Beckman Coulter, Nyon Switzerland) anti-human CD68 (macrophages, diluted: 1:100; Dako Corporation) and anti-human matrix metalloproteinase (MMP)-9 (diluted 1:250; Southern Biotech, Birmingham AL).

Quantifications were performed using MetaMorph™ 6 software. Data were presented as cells/mm2 (neutrophils) or percentages of the stained area on total lesion area (other parameters).

Statistical analyses

Patient characteristics were described one day before endarterectomy (**Table 1**). Categorical data are presented as relative and absolute frequencies. Continuous variables were expressed as median and interquartile range (IQR) as the normality assumption was not demonstrated.

Intergroup comparisons were drawn by Fisher's exact test and Mann-Whitney U-test, as appropriate. Ranked Spearman correlation coefficients were performed to establish correlations between Lp(a) serum levels and inflammatory biomarkers in both upstream and downstream portions of atherosclerotic plaques. Kaplan-Meier analysis was performed to estimate cumulative ACS rate at follow-up and to calculate the corresponding risk difference according to the study groups. The effect of Lp(a) ≥10 mg/dL on the risk of the ACS was estimated by Cox proportional hazard models and expressed with hazard ratios (HR) and 95% CI. Multivariate analysis was performed adjusting for some classical risk factors for ACS, such as gender, dyslipidaemia and coronary artery disease (CAD), in order to obtain estimates of the HRs and to identify factors independently associated with the outcome. Values with $p<0.05$ (two-tailed) were considered significant. All analyses were performed with the SPSS statistical software, release 23.0 ® (IBM CO., Armonk, NY).

Results

Patients' characteristics

Baseline demographic, clinical and biochemical characteristics, as well as medications of the patients according to the Lp(a) threshold value of 10 mg/dL, are shown in **Table 1**. As potentially expected, patients with high Lp(a) levels at admission were more affected by chronic coronary artery disease (18.9 vs. 15.0%; p=0.021), higher d-dimer plasma levels (266.2 vs. 179.4 μg/mL; p=0.003), as well as higher serum levels of fibrinogen (4.19 vs. 3.63 mg/dL, p=0.016).

Table 1. Clinical characteristics of study groups.

	Lp(a)*** <10 mg/mL (n=140)	Lp(a) ≥10 mg/mL (n=40)	*p*-value
Demographic			
Age, yr. (IQR)	72 (67-77)	74 (68-78)	0.290
Males, no. (%)	46 (63.5)	21 (63.6)	0.840
Symptomatic, no. (%)	30 (21.4)	4 (10.0)	0.115
Systolic BP*, mmHg (IQR)	135 (130-140)	130 (125-150)	0.924
Diastolic BP, mmHg (IQR)	80 (80-90)	80 (80-90)	0.581
Waist circumference, cm (IQR)	93 (87-98)	90 (88-95)	0.714
Carotid stenosis, % (IQR)	80 (70-90)	75 (70-80)	0.132
Hypertension, no. (%)	100 (71.4)	26 (65.0)	0.440
Active smokers, no. (%)	29 (20.7)	11 (27.5)	0.391
Previous smokers, no. (%)	67 (47.9)	14 (35.0)	0.207
Type 2 diabetes, no. (%)	32 (2.9)	6 (15.0)	0.380
Dyslipidaemia, no. (%)	79 (56.4)	22 (55.0)	1.000
Chronic CAD†, no. (%)	21 (15.0)	13 (18.9)	**0.021**
Medications			
RAAS‡ inhibitors, no. (%)	67 (47.9)	21 (52.5)	0.720
ACE-I§, no. (%)	7 (5.0)	4 (10.0)	0.265
ARBs‖, no. (%)	60 (42.9)	17 (42.5)	1.000
β-blockers, no. (%)	42 (30.0)	9 (22.5)	0.429
Calcium antagonists, no. (%)	40 (28.6)	14 (35.0)	0.440
Statins, no. (%)	65 (46.4)	24 (60.0)	0.153
Aspirin, no. (%)	77 (55.0)	25 (62.5)	0.471
Thienopyridine, no. (%)	29 (20.7)	8 (20.0)	1.000
Anticoagulants, no. (%)	9 (6.4)	0 (0.0)	0.210
Oral antidiabetics, no. (%)	18 (12.9)	5 (12.5)	1.000
Insulin, no. (%)	5 (3.6)	0 (0.0)	0.588

Haematology			
Total WBC[#], no. x 10^9/L (IQR)	6.93 (6.28-8.10)	7.45 (6.03-8.41)	0.371
Neutrophil, no. x 10^9/L (IQR)	4.37 (3.55-5.30)	4.74 (3.70-5.96)	0.170
Lymphocyte, no. x 10^9/L (IQR)	1.80 (1.42-2.19)	1.75 (1.55-2.06)	0.836
Monocyte, no. x 10^9/L (IQR)	0.43 (0.34-0.56)	0.47 (0.41-0.54)	0.283
Platelet, no. x 10^9/L (IQR)	231 (187-286)	225 (188-266)	0.388
Red blood cell, no. x 10^{12}/L (IQR)	4.6 (4.4-4.9)	4.6 (4.2-5.00)	0.640
Chemistry			0.059
Fasting glycaemia, mg/dL (IQR)	101 (90-164)	116 (92-176)	0.215
Serum total-c[**] mg/dL (IQR)	191 (163-219)	201 (177-240)	0.054
Serum LDL-c[††] mg/dL (IQR)	112 (83-140)	121 (103-161)	0.888
Serum HDL-c[‡‡] mg/dL (IQR)	48 (41-61)	48 (40-61)	0.467
Serum TAG[§§] mg/dL (IQR)	118 (89-166)	122 (92-179)	0.215
Fibrinogen, mg/dL (IQR)	3.63 (3.14-4.15)	4.19 (3.28-5.05)	**0.016**
D-dimer, µg/mL (IQR)	179.4 (106.3-343.9)	266.2 (150.1-1422.0)	**0.003**
hsCRP[##], µg/mL (IQR)	2.05 (0.81-4.67)	2.75 (0.50-6.52)	0.504

Data are expressed as median (interquartile range [IQR]) or number [no.] (percentages [%]).

Pearson χ2 test or Fisher exact test were used for comparison of qualitative variables and Mann-Whitney non-parametric test for comparisons of continuous variables.

* BP: blood pressure

† CAD: coronary artery disease

‡ RAAS: renin-angiotensin-aldosterone system.

§ ACE-I: angiotensin converting enzyme inhibitor

|| ARBs: angiotensin receptor blockers

WBC: white blood cells

** total-c: total cholesterol

†† LDL-c: low-density lipoprotein cholesterol

‡‡ HDL-c: high-density lipoprotein cholesterol

§§ TAG: triglyceride

hsCRP: high-sensitivity C-reactive protein

*** Lp(a): Lipoprotein(a)

Patients with high and low serum levels of Lp(a) have a similar histological composition of carotid plaques

When histological intraplaque parameters were assessed within different portions (upstream and downstream the blood flow) of carotid plaques, patients with high Lp(a) levels had similar lipid, collagen, SMC, macrophage,

neutrophil and MMP-9 content with respect to patients with low levels (**Table 2**).

Table 2. Distribution of intraplaque biomarkers in patients with high and low Lp(a) levels.

	Lp(a)‡ <10 mg/dL (n=140)	Lp(a) ≥10 mg/dL (n=40)	*p*-value
Upstream portion			
Lipids, %	5.87 (2.81-10.56)	5.47 (2.76-9.87)	0.847
Total collagen, %	30.70 (18.39-38.38)	25.38 (17.24-39.33)	0.715
Type I collagen, %	10.40 (6.16-15.29)	10.10 (5.26-14.45)	0.812
Type II collagen, %	12.98 (8.69-18.26)	12.19 (7.47-17.69)	0.506
Total SMC*, %	4.54 (2.84-9.66)	6.78 (4.13-12.18)	0.052
Total macrophages, %	5.34 (2.63-10.32)	5.55 (2.95-9.53)	0.801
Total neutrophils, cells+/mm^2	2.03 (0.76-5.08)	2.36 (0.91-5.72)	0.822
MMP†-9, %	2.98 (0.81-7.39)	5.23 (1.22-10.29)	0.275
Downstream portion			
Lipids, %	4.30 (1.88-8.39)	4.26 (1.67-8.20)	0.847
Total collagen, %	17.9 (9.08-22.21)	13.29 (7.01-20.70)	0.229
Type I collagen, %	5.47 (2.88-10.31)	5.47 (3.20-7.78)	0.667
Type II collagen, %	5.70 (3.32-9.72)	5.48 (3.16-9.28)	0.783
Total SMC, %	2.77 (1.59-4.12)	2.38 (1.87-5.01)	0.950
Total macrophages, %	6.19 (2.26-11.33)	5.88 (1.94-16.23)	0.989
Total neutrophils, cells+/mm^2	3.70 (1.19-10.64)	5.77 (0.67-11.47)	0.919
MMP-9, %	5.78 (2.82-15.93)	4.88 (2.36-15.39)	0.527

Data are expressed as median (interquartile range).

Comparisons were drawn by Mann-Whitney test.

* SMC: smooth muscle cell.

† MMP: matrix metalloproteinase.

‡ Lp(a): Lipoprotein(a).

Also, when assessing the potential association between Lp(a) levels and intraplaque parameters, only a weak positive association between SMC and Lp(a) levels was found in upstream regions (**Table 3**). Taken together, these results suggest that Lp(a) serum levels were not associated with histological features of intraplaque carotid inflammation.

Table 3. Correlation between serum levels of Lp(a) and intraplaque parameters.

	Lp(a)‡, mg/dL	
	r	*p*-value
Upstream portion		
Lipids, %	-0.041	0.622
Total collagen, %	0.048	0.560
Type I collagen, %	0.055	0.501
Type II collagen, %	-0.005	0.954
Total SMC*, %	0.195	**0.017**
Total macrophages, %	-0.025	0.761
Total neutrophils, cells+/mm^2	-0.033	0.686
MMP†-9, %	0.027	0.747
Downstream portion		
Lipids, %	0.040	0.622
Total collagen, %	-0.102	0.210
Type I collagen, %	-0.061	0.457
Type II collagen, %	-0.070	0.387
Total SMCs, %	-0.057	0.487
Total macrophages, %	-0.024	0.771
Total neutrophils, cells+/mm^2	0.077	0.348
MMP-9, %	-0.081	0.321

Correlations were performed by Spearman's rank correlation coefficient.

* SMC: smooth muscle cell.

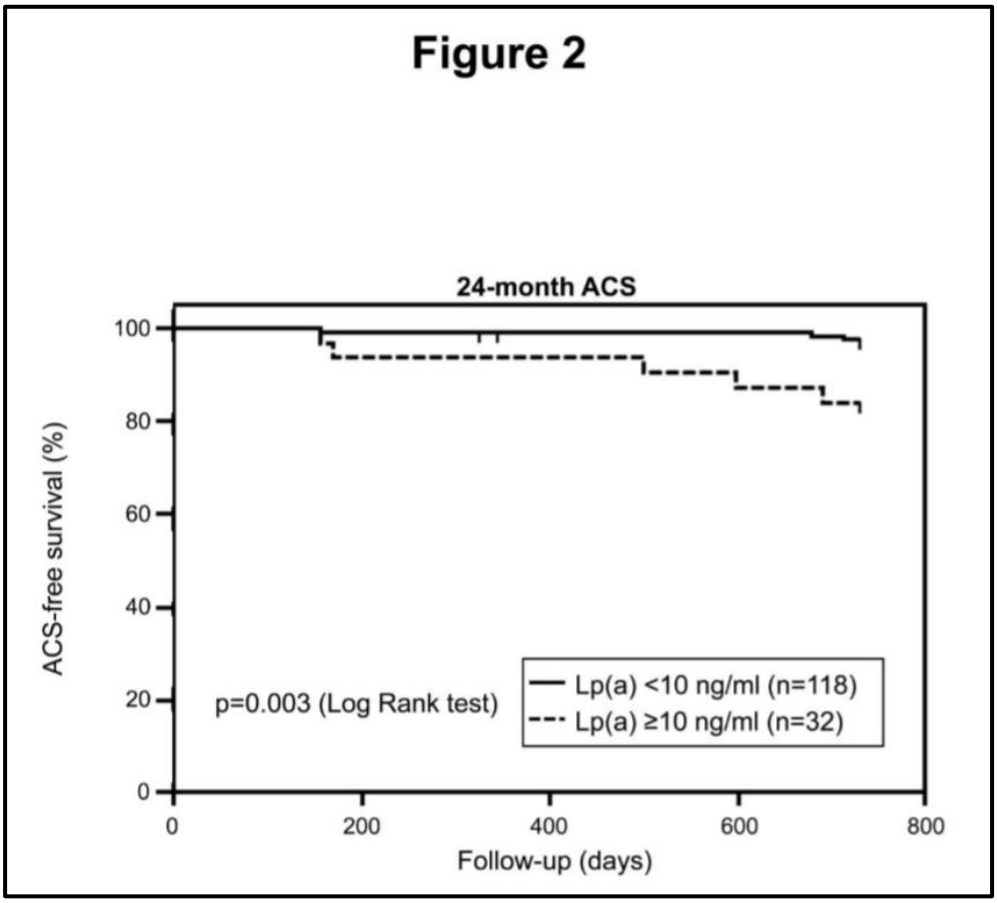
† MMP: matrix metalloproteinase.

‡ Lp(a): Lipoprotein(a).

3.3 Serum levels of Lp(a) ≥10 mg/dL predict ACS at 24-month follow-up

Based on a Kaplan-Meyer curve analysis, patients with Lp(a) serum levels ≥10 mg/dL were shown to develop more ACS at 24-month follow-up as compared to low Lp(a) serum levels (12.5% vs 2.1%, Log Rank test: p=0.003, **Figure 2**).

Figure 2



Importantly, Lp(a) serum levels ≥10 mg/dL were able to predict ACS at 24-month follow-up (HR: 6.490 [95% CI: 1.550-27.160]; p=0.010) (Table 4). This association remained statistically significant after adjustment for known cardiovascular risk factors, such as male gender, dyslipidemia and chronic CAD (adjusted HR: 8.504 [95% CI: 1.932-37.425]; p=0.005) **(Table 4).**

Table 4. Cox hazard proportional model showing the predictive value of categorized Lp(a) levels towards the occurrence of acute coronary syndrome at 24-month follow-up.

	Univariate model			Multivariate model		
	HR*	95% CI†	*p*-value	HR	95% CI	*p*-value
24-month ACS‡						
Lp(a)§ ≥10 mg/dL	6.490	1.550-27.160	**0.010**	8.504	1.932-37.425	**0.005**
Gender, male	0.583	0.146-2.331	0.445	1.093	0.193-6.184	0.920
Dyslipidaemia	2.684	0.558-12.920	0.218	6.352	0.740-54.498	0.092
CAD‖	1.518	0.302-7.621	0.612	1.506	0.281-8.074	0.633

* HR: hazards ratio.

† CI: confidence interval.

‡ ACS: acute coronary syndrome.

§ Lp(a): Lipoprotein(a).

‖ CAD: coronary artery disease.

Discussion

The main finding of this pilot analysis is that in patients with severe carotid artery stenosis (advanced atherosclerosis), Lp(a) serum levels ≥10 mg/dL were associated with an increased risk of developing an ACS. Circulating concentrations of Lp(a) were reported to range from <0.1 mg/dL to more than 200 mg/dL in healthy individuals [7], independently of age and gender. It is estimated that about 70% of the variance of Lp(a) concentrations are genetically set, mainly by the LPA gene, which determines both the synthetic hepatic rate and the size of the apopoprotein(a) within Lp(a) [21]. Once released in the systemic circulation, elevated Lp(a) levels have been associated with CVD risk [22], suggesting that a genetic predisposition to high Lp(a) levels might significantly impact on accelerated atherogenesis [23]. Since this observation was mainly reported in primary prevention studies, we focused on the potential prognostic role of high Lp(a) levels on ACS in patients with advanced atherosclerosis. A systematic review of secondary prevention studies recently confirmed a weak, but still significant association between Lp(a) levels and future CV events [10].

However, when considering cerebrovascular events, no significant association between elevated Lp(a) levels and recurrent stroke or transient ischemic attack was found [24]. This is in line with results of the Atherosclerosis Risk in Communities (ARIC) study [25], showing that elevated Lp(a) levels were mainly associated with an increased risk of CAD, but not of stroke. Another relevant issue of our study is represented by the identification of pathologic ranges of serum Lp(a) levels in patients with advanced atherosclerotic burden [13]. Also, due to different methods of

measurement, Lp(a) cut-off values are quite heterogeneous in current publications [26]. For instance, Lp(a) levels ranging from 10 to 77 mg/dL were indicated to predict cardiovascular events [10, 19, 23]. In our study investigating patients with severe carotid stenosis, we focused on the lowest Lp(a) level (10 mg/dL) reported as a potential cut-off value, in order to assess the prognostic accuracy of this parameter to predict ACS. This selection allowed us to demonstrate that Lp(a) levels above 10 mg/dL were predictive of the development of ACS at two-year follow-up. These data may suggest to use a quite low cut off level for Lp(a) in patients with advanced atherogenesis. Considering carotid intraplaque characteristics, no difference was found between patients with the high and low serum levels of Lp(a).

Furthermore, only a weak positive association between serum Lp(a) levels and intraplaque SMC content in upstream regions was found, suggesting no strong association of Lp(a) concentrations and intraplaque inflammation. Our results are in line with previous evidence of a modest impact of Lp(a) levels on carotid plaque morphology. For instance, no association between the Lp(a) and carotid intima-media thickness (IMT) in different populations was reported [27-31]. These observations indicate a high heterogeneity of atherosclerotic plaques at different arterial districts (i.e. carotid vs coronary atherosclerotic plaques). Evidence from the present investigation and other translational studies may support a specific role for Lp(a) in coronary rather than carotid atherosclerosis [32]. Clinical interventional studies with Lp(a) lowering treatments potentially reducing coronary events are needed to corroborate our observation further. The present study has some limitations. Firstly, we performed a pilot study in which the cut-off value of Lp(a) was

selected from recently published clinical studies [19]. Therefore, we risked to miss significant differences between groups due to potential low study power. Secondly, although the Lp(a) measurement was obtained using an isoform-insensitive nephelometry assay, we did not assess apoprotein(a) isoforms. Apoprotein(a) size isoforms are inversely correlated with Lp(a) concentration and low-molecular-weight apo(a) patterns have been reported as independent risk factors for atherosclerosis [11, 33]. Therefore, the evaluation of the apo(a) genotype would have allowed to better understand the potential biological relation of Lp(a) and ACS. Thirdly, given the little number of outcomes, the predictive model of Lp(a) for 24 months ACS could be adjusted only with a limited number (n=3) of independent variables.

CONCLUSIONS

In conclusion, we showed that a low cut-off level of Lp(a) concentration ≥10 mg/dL can predict an ACS event in high CVD risk population with severe carotid atherosclerosis. Despite the potential low study power, these data may suggest to use a quite low cut-off level for Lp(a) in patients with advanced atherogenesis. When the histological parameters of the carotid plaques were taken into account, we did not find a difference between patients with the high and low serum levels of Lp(a). The weak positive association between Lp(a) levels and intraplaque SMC that our data highlighted suggest no strong association of Lp(a) concentrations and intraplaque inflammation. Our results are in line with others translational studies, indicating a high heterogeneity of atherosclerotic plaques at different vascular districts, supporting the hypothesis of a specific role for Lp(a) in coronary rather than carotid atherosclerosis.

Finally, there are currently no approved pharmacologic therapies that specifically target lipoprotein(a). Despite this, preclinical [34, 35] as well as phase I and phase II studies on antisense oligonucleotides inhibiting the production of the apolipoprotein(a) in the hepatocyte [36-38], showed a reduction of Lp(a) levels till 80% in patients who had established cardiovascular disease [38]. Hence, our results might be interesting to define future therapeutic targets better.

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