Lipoprotein(a), fibrinogen and vascular mortality in an elderly northern Italian population

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Background and Objectives. High lipoprotein a [Lp(a)] and fibrinogen levels are suggested risk factors for coronary heart disease (CHD) and stroke morbidity and mortality. Experimental data strongly suggest that the mechanisms of atherothrombosis include an interaction between fibrinogen and Lp(a), but little clinical evidence of a synergism between these two parameters has been reported.

Design and Methods. Within the frame of a prospective population study conducted in the area of Cremona (Lombardy, Italy), 343 women and 216 men aged ≥65 years were evaluated for clinical and biochemical cardiovascular risk factors. Lp(a) levels ≥30 mg/dL were observed in 22.7% and 23.9% of men and women, respectively. Fibrinogen levels were higher in women (p<0.0001). After a median follow-up of 6.3 years 107 deaths were recorded, of which 33 were due to CHD or ischemic stroke.

Results. The combined incidence rate of CHD and stroke mortality increased from 10.8 (per 1000 person-years) for subjects with either Lp(a) ≥30 mg/dL or fibrinogen within the 5th quintile of the gender-specific distribution to 38.4 for subjects with both Lp(a) ≥30 mg/dL and fibrinogen within the 5th quintile. Age (p<0.0001), insulin (p<0.0002) and the combination of high Lp(a) and fibrinogen (hazard ratio=3.11, p=0.014), but not fibrinogen or Lp(a) levels in isolation, were independent predictors of CHD and stroke mortality. In a subgroup of 447 subjects in whom C-reactive protein (CRP) was measured, CRP levels were not predictive of combined CHD and stroke mortality.

Interpretations and Conclusions. Based on these results obtained in a relatively small population of elderly subjects, the association of high Lp(a) and fibrinogen levels appears to carry an increased risk of pooled CHD and stroke mortality.

Key words: coronary heart disease, stroke, Lp(a), fibrinogen, insulin, C-reactive protein.

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A recent report has renewed attention to lipoprotein [Lp(a)] as an independent predictor of vascular mortality among older men.[1] Lp(a) is a low-density lipoprotein particle in which apolipoprotein B-100 is linked to apoprotein(a).[2] The size of apolipoprotein(a), under genetic control, ranges between 300 and 800 kDa, due to differences in the number of kringle IV type 2 repeats,[3] and the smaller isoforms are associated with elevated plasma Lp(a) levels and a higher cardiovascular risk.[4,5] Increased fibrinogen levels are also a recognized risk factor for cardiovascular disease,[6,7] and experimental data strongly suggest that a fibrinogen-Lp(a) interaction is involved in the mechanisms of atherothrombosis. High Lp(a) levels may be prothrombotic by impairing fibrinolysis.[8] Lp(a) alters the structure of the fibrin network decreasing clot permeability and clot lysis in plasma.[9] Accumulation of Lp(a) in atherosclerotic plaques is mediated through an interaction of fibrin deposits with the apolipoprotein(a) moiety of Lp(a) and there is evidence for mechanisms bridging Lp(a) to places of fibrin deposition such as injured vessels or atherosclerotic lesions.[10] Fibrinogen deficiency in apo(a) transgenic mice reduces accumulation of apo(a) in the vessel walls and lesion development.[11] These data suggest that elderly subjects with high levels of both Lp(a) and fibrinogen levels could be at significant risk of cardiovascular events.

In this study, we prospectively evaluated risk factors for coronary heart disease (CHD) mortality and stroke mortality in elderly subjects from an Italian province.

Design and Methods

A population study was conducted in the area of Cremona (Lombardy, Italy) during the years 1990-91. This study was mainly focused on estimating the prevalence of diabetes mellitus and impaired carbohydrate tolerance, but great attention was also given to the collection of information regarding clinical and laboratory markers of cardiovascular risk. A sample of 3,597 subjects aged 40-87 years was identified. These subjects

were invited to undergo a medical evaluation in three outpatient clinics over a period of 3 months. The study was approved by the *H S. Raffaele* Institutional Review Board and subjects gave informed consent. Blood samples were taken from all subjects in the fasting state and after an oral glucose load (75 g) and then the patients were evaluated by attending physicians. Blood pressure (on two separate occasions), morphometric data and a standard questionnaire regarding lifestyle (including smoking habits and alcohol ingestion), drug consumption and work were recorded for each subject. The physicians and instruments were always the same for the entire duration of the study, and all blood samples for standard biochemical evaluation [(blood glucose at baseline and 2 hours after the glucose load, alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyltransferase (γ-GT)] were analyzed by a single laboratory in Cremona. In addition, aliquots of citrated plasma from each subject, obtained by centrifugation *in situ*, were snap-frozen and stored at –70°C in our Institution. A total of 2,096 subjects participated in the study (58% of those invited) with no apparent selection for sex and age. A family history of diabetes had a minor influence on participation, but no evidence of a selection bias was apparent for any other characteristics. Participants were eligible for enrollment whether or not they had a history of vascular disease.

In March 1997 information was collected on causes of any deaths that had occurred in all the subjects since January 1991. Information about deaths was obtained from reviews of Regional Health Registry files, medical records, death certificates, and autopsy reports. Causes of death were classified using the International Classification of Diseases, Eight and Ninth Revision, codes 410-414 (coronary heart disease, CHD), 430-438 (stroke). Some details related to this population have been previously reported.[12]

Diabetes status was classified according to WHO criteria.[13] A subject was considered to have metabolic syndrome if three or more of the following categories were present: (i) waist circumference >102 cm in men and >88 cm in women; (ii) systolic blood pressure >130 mmHg or diastolic blood pressure > 85 mmHg or use of antihypertensive treatment; (iii) triglyceride levels >150 mg/dL; (iv) high density lipoprotein (HDL) cholesterol levels <40 mg/dL in men or <50 mg/dL in women; and (v) fasting plasma glucose >110 mg/dL.[14]

Total and HDL-cholesterol, triglycerides (Boehringer Mannheim, Germany), Lp(a) (TintElize Lp(a), Biopool, Umea, Sweden), insulin (Technogenetics, Medgenics, Belgium) and fibrinogen (Boehringer) levels were assayed at our Institution after an average interval of 0.9 years from blood collection on snap-frozen citrated plasma samples stored at –70°C and never thawed before. The enzyme-linked immunosorbent assay (ELISA) for Lp(a) uses polyclonal antibodies to human apo(a) both as catching and detecting antibodies and detects free apo(a) as well as Lp(a) isoforms. In 2004, C reactive protein (CRP) levels (N high-sensitivity CRP, Dade-Behring, Marburg Germany) were measured by a technician blinded with respect to the end-point of the study in citrated plasma samples still available from 447 subjects These samples had not been previously thawed.

Statistical analysis

In view of the low mortality rate among the younger subjects (all-cause mortality=12.1 per 1000 person-years, CHD + stroke mortality=0.97 per 1000 person-years), the analysis was conducted on 559 subjects aged 65 years or more at enrollment (47.5% of the invited subjects). The distribution of fibrinogen values was stratified into quintiles, separately for men and women, with the cut-off points chosen so that each quintile group contained approximately 20% of the full cohort. A cut-off value of 30 mg/dL was chosen for the definition of increased Lp(a) levels. Incidence rates were calculated as the number of events per 1000 person-years at risk. Curves for overall survival or event-free survival were estimated using the Kaplan-Meier method. Potential confounders were defined as traditional risk factors or variables that were significantly associated with Lp(a) or fibrinogen levels. Because the frequency distributions of Lp(a), fibrinogen, insulin, CRP and liver function markers were highly skewed, the associations between the log levels of these parameters and continuous variables were assessed using Pearson's correlation coefficients, and the associations between the log levels of these parameters and categorical variables were assessed using a t-test or analysis of variance. Cox univariate proportional-hazards models,[15] including gender and age as covariates, were used to assess the age and sex adjusted hazard ratio associated with the events for relevant single laboratory and clinical risk factors. Subjects with Lp(a) ≥30 mg/dL, with fibrinogen levels within the 5th quintile of the gender-specific fibrinogen distributions, with either Lp(a) levels ≥30 mg/dL or fibrinogen levels within the 5th quintile of the gender-specific distributions, or with both Lp(a) levels greater than 30 mg/dL and fibrinogen levels within the 5th quintile of the gender-specific distributions were compared to the remaining subjects. The multivariate Cox proportional model (stepwise) including parameters with p values <0.1 at univariate analysis was used to investigate the independent association of the risk factors with event-related mortality. Hazard ratios (HR) and 95% confidence intervals (95% CI) are reported.

p values for all tests were two-tailed, and differences were considered to be statistically significant at the 0.05 level. HR significant at a level of p<0.1 are shown. Statistical analyses and plots were performed with

Systat 7.0 for Windows software (SPSS Inc, Chicago, IL, USA).

Results

The population of subjects aged 65 years or more at enrollment consisted of 343 women and 216 men (Table 1). The age at entry ranged from 65 to 87 years and was higher for women than for men. Men were more likely then women to be current or former smokers and to drink more alcohol. Hypertension and depression or anxiety syndrome were more frequently recorded in women than in men. Women had higher total, low density lipoprotein (LDL) and HDL cholesterol and alkaline phosphatase levels than men, but lower levels of AST, ALT and γ-GT. In spite of higher post-load glucose levels in women than in men, the prevalence of diabetes and impaired glucose tolerance were similar in men and women. The metabolic syndrome was observed more frequently in women than in men and was detected in 70.4% of subjects with diabetes, in 75.4% of subjects with impaired glucose tolerance and in 19.5% of the remaining subjects (p<0.0001). There was a history of ischemic heart disease, cerebrovascular disease or peripheral artery occlusive disease in 41.8% of subjects with the metabolic syndrome and in 32.8% of those without (p=0.05).

Lp(a) levels were not significantly different in men and women (Figure 1, panel A), and correlated with total and LDL cholesterol levels (r≥0.185, p≤0.001). Lp(a) levels ≥30 mg/dL were observed in 49 men (22.7%) and 82 women (23.9%, p=0.74). Fibrinogen levels were higher in women than in men (Figure 1, right panel) and were significantly and inversely correlated with daily alcohol ingestion (r=-0.195, p=0.0002), AST (r=-0.151, p=0.014) and γ-GT levels (r=-0.150, p=0.015). Cut-off values for the 5th gender-specific quintiles were 370 mg/dL in women and 341 mg/dL in men, with the 5th quintiles including 21.3% and 21.0% of men and women, respectively. Fibrinogen and Lp(a) levels did not show significant associations with blood pressure, diabetes, impaired glucose tolerance, history of ischemic heart disease, cerebrovascular disease or peripheral artery occlusive disease (p≥0.12). Fibrinogen levels were higher in subjects with metabolic syndrome (310 mg/dL and 510 mg/dL, median and range) than in the remaining subjects (281 mg/dL and 561 mg/dL, p<0.0001), with 30.1% of subjects with metabolic syndrome having fibrinogen levels within the 5th quintile of the distribution (p=0.001). Lp(a) levels were similar in subjects with the metabolic syndrome (10.9 mg/dL and 154.2 mg/dL) and without (12.7 mg/dL and 157.1 mg/dl, p=0.11); Lp(a) levels ≥30 mg/dL were observed in 22.5% of subjects with the metabolic syndrome (p=0.69).

After a follow-up of 3,238 person-years (median follow up 6.3 years), a total of 107 subjects had died, of whom 16 due to CHD and 17 due to ischemic stroke. All-cause, CHD and stroke mortality rates were 33.0, 4.9 and 5.3 per 1000 person-years, respectively. Incidence rates for each event (the number of events per 1000 person-years at risk) are shown separately for men and women and for the entire cohort, according to Lp(a)

Table 1. Baseline characteristics of the study cohort.

Variable	*Entire cohort (n=559)*	*Women (n=343)*	*Men (n=216)*	*p*
Age (years)	71.4±5.2	71.9±5.3	70.7±4.9	0.015
Fasting glucose (mg/dL)	99.4±22.9	100.1±25.0	98.3±19.1	0.53
Post-load glucose (2 h, mg/dL)*	118.9±52.9	125.8±53.9	108.2±49.6	0<.0001
Insulin (mU/L)	15 (177)	15 (148)	14 (177)	0.13
Blood pressure (mmHg)				
Diastolic	81.1±12.7	80.3±13.2	82.2±11.9	0.059
Systolic	155.5±22.1	156.0±22.6	154.8±21.3	0.61
Cholesterol (mg/dL)				
Total	236.8±42.7	245.3±42.7	223.2±39.1	<.0001
LDL	158.7±39.5	164.9±39.3	148.7 ± 37.8	<.0001
HDL	52.5±15.2	54.6±14.5	49.0 ± 15.6	<.0001
Triglycerides (mg/dL)	130.0±59.6	130.2±59.0	129.6 ± 60.8	0.62
Body-mass index (kg/m^2)	26.8±4.3	26.8±4.6	26.7 ± 3.8	1.00
Former or current smoking (%)	34.7	9.3	75.0	<0.0001
On hypotensive drugs (%)	37.9	41.7	31.9	0.01
Diabetes status (%)†				
Normal	70.5	67.3	75.5	
Impaired glucose tolerance	10.2	11.4	8.3	0.39
Diabetes	19.3	21.3	16.2	0.20
Metabolic syndrome‡	35.1	41.1	25.5	0.0002
History of IHD and/or CVD (%)	17.9	19.5	15.3	0.21
Peripheral artery occlusive disease (%)	7.3	6.7	8.3	0.79
Depression/anxiety symdrome (%)	4.7	6.4	1.9	0.02
Gastritis/gastric ulcer (%)	7.5	6.7	8.8	0.58
Liver disease (%)	1.25	1.45	0.93	0.58
History of cancer (%)	1.8	1.2	2.8	0.28
Alcohol ingestion > 30 g/day (%)	46.3	29.0	73.8	<0.0001
Coffee intake (%)	79.9	81.0	78.1	0.41
Regular drug intake (%)	61.1	67.6	65.1	0.12
Estrogen use (%)	–	3.5	–	
AST (U/L)	25 (105)	24 (97)	26 (105)	0.036
ALT (U/L)	18 (160)	17 (95)	19 (160)	0.008
γ-GT (U/L)	24 (996)	20 (996)	29 (622)	<0.0001
ALP (U/L)	185 (991)	193 (946)	175 (739)	<0.0001
Lp(a) lipoprotein (mg/dL)	12.2 (157.1)	12.2 (154.2)	12.0 (157.1)	0.60
Fibrinogen (mg/dL)	295.0 (561)	310.0 (540)	272.5 (531)	<0.0001
C reactive protein (mg/L)§	1.34 (43.15)	1.43 (29.15)	1.19 (43.15)	0.21

*Means ± SD and median and (range) are reported for continuous variables normally and not normally distributed. LDL: low-density lipoprotein; HDL: high-density lipoprotein. The body-mass index is the weight in kilograms divided by the square of the height in meters. *Determined in 253 women and 164 men. †Diabetes status was classified according to the criteria of the WHO.[13] ‡ Defined according to ATP-III criteria.[14] IHD: ischemic heart disease; CVD: cerebrovascular disease. §Determined in 266 women and 181 men.*

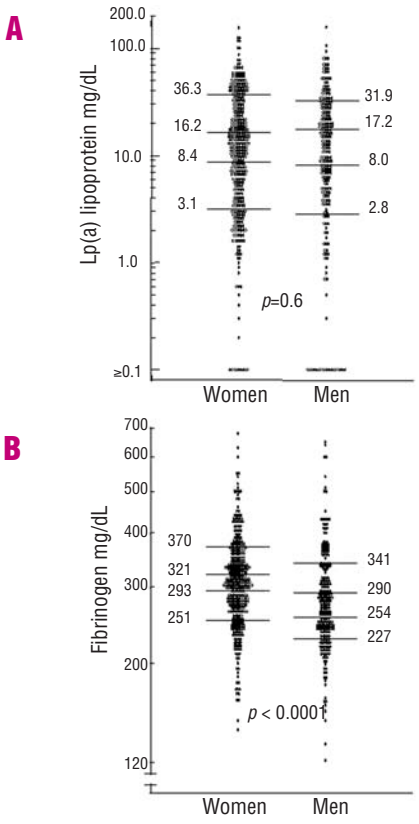

Figure 1. Distribution of Lp(a) (left panel) and fibrinogen (right panel) levels according to gender in the study cohort. Lp(a) and fibrinogen levels are on a logarithmic scale and cut-off values for quintiles 2 to 5 are shown. Fibrinogen levels, but not Lp(a) levels, were significantly higher in women than in men.

and fibrinogen levels, in Table 2. While all cause-mortality was not influenced by either Lp(a) or fibrinogen levels, CHD and stroke mortality were roughly two times greater in both women and men with Lp(a) levels ≥30 mg/dL. Similarly, the combined CHD and stroke mortality was about two times higher in men and women in the 5th quintile of the fibrinogen distribution than in the remaining subjects. In both men and women there was a clear effect of the association of high Lp(a) and fibrinogen levels on the combined CHD and stroke mortality. In the entire cohort, the combined incidence rate of CHD and stroke mortality increased from 7.6 per 1000 person-years for subjects with Lp(a) levels <30 mg/dL and fibrinogen levels in the four lower quintiles of the distribution to 38.4 per 1000 person-years for the 30 subjects with both Lp(a) levels ≥30 mg/dL and fibrinogen levels in the 5th quintile of the gender-specific distribution (Table 2).

In univariate Cox models, all-cause mortality was positively associated with age (HR=1.15; 95%CI: 1.11-1.19 for each year of age, $p<0.0001$) and male gender (HR=1.72; 95%CI: 1.18-2.52, $p=0.004$). When adjusting for age and gender, liver disease and AST, ALT, fasting glucose and insulin levels were positively associated with all-cause mortality (Table 3). Lp(a) levels ≥30 mg/dL, fibrinogen levels in the 5th quintile of the gender-specific distributions and the combination of high Lp(a) and fibrinogen levels resulted in non-significant hazard ratios. Adjusting for age and gender, the combined CHD and stroke mortality was positively associated with diabetes, but not with the metabolic syndrome

Table 2. Incidences of all-cause mortality and of mortality due to coronary heart disease (CHD) and stroke according to gender, Lp(a) and fibrinogen levels at baseline.

	Entire cohort					*Women* no. of deaths/1000 person-years					*Men*				
	n	*All-cause*	*CHD*	*Stroke*	*CHD + Stroke*	*n*	*All-cause*	*CHD*	*Stroke*	*CHD + Stroke*	*n*	*All-cause*	*CHD*	*Stroke*	*CHD + Stroke*
Lp(a)															
<30 mg/dL	428	32.7	4.0	4.0	8.1	261	24.8	2.6	3.3	5.9	167	45.5	6.4	5.3	11.7
≥30 mg/dL	131	34.3	7.9	9.2	17.1	82	29.7	10.6	6.4	17.0	49	41.9	3.5	14.0	17.5
Fibrinogen*															
Quintiles 1-4	441	32.2	3.5	5.0	8.5	271	25.7	3.1	4.4	7.5	170	42.8	4.1	6.1	10.2
5th Quintile	118	36.2	10.6	6.0	16.6	72	26.7	9.7	2.4	12.1	46	52.0	12.0	12.0	24.0
Lp(a) <30 mg/dL and fibrinogen in quintiles 1-4	340	33.4	3.5	4.1	7.6	204	26.8	2.5	4.2	6.7	136	43.8	5.2	3.9	9.0
Lp(a) ≥30 mg/dL or fibrinogen in 5th quintile	189	28.9	4.5	6.3	10.8	124	20.4	4.1	2.7	6.8	65	45.7	5.4	13.4	18.8
Lp(a) ≥30 mg/dL and fibrinogen in 5th quintile	30	57.6	25.6	12.8	38.4	15	67.3	40.4	13.5	53.8	15	48.8	12.2	12.2	24.4
Total	559	33.0	4.9	5.3	10.2	343	25.9	4.5	4.09	8.5	216	44.7	5.7	7.3	13.0

**Gender-specific quintiles (5th quintile: ≥370 mg/dL in women and ≥341 mg/dL in men).*

Table 3. Hazard ratios (95% confidence intervals) for combined CHD and stroke mortality and for all-cause mortality: univariate Cox models adjusted for age and gender.

	CHD+stroke mortality	p	All-cause mortality	p
Liver disease	6.80 (0.89-52.0)	0.06	6.60 (2.05-21.5)	0.002
AST (U/L)	0.99 (0.95-1.02)	0.47	1.025 (1.01-1.04)	<0.0001
ALT (U/L)	0.99 (0.96-1.02)	0.44	1.02 (1.005-1.023)	0.001
γ-GT (U/L)	1.00 (0.99-1.00)	0.71	1.001 (1.00-1.003)	0.08
Diabetes	2.18 (1.03-4.60)	0.04	1.48 (0.91-2.40)	0.11
Fasting glucose (mg/dL)	1.01 (1.003-1.03)	0.009	1.007 (1.00-1.01)	0.04
Insulin (mU/L)	1.03 (1.01-1.04)	<0.0001	1.02 (1.01-1.03)	<0.0001
Triglycerides (mg/dL)	1.006 (1.002-1.01)	0.007	1.00 (1.00-1.00)	0.43
Coffe intake	0.50 (0.24-1.04)	0.06	0.68 (0.44-1.04)	0.08
Lp(a) ≥30 mg/L	1.89 (0.95-3.85)	0.08	0.95 (0.61-1.49)	0.10
Fibrinogen in 5th quintile	1.68 (0.83-3.57)	0.16	0.98 (0.63-1.59)	0.10
Lp(a) ≥30 mg/L and Fibrinogen 5th quintile	3.42 (1.39-8.42)	0.008	1.47 (0.74-2.93)	0.10

Hazard ratios for continuous variables are reported for unitary increase. In addition to Lp(a) and fibrinogen, only variables with p <0.1 for CHD and stroke mortality or all-cause mortality are shown.

Table 4. Hazard ratios (95% confidence intervals) for combined CHD and stroke mortality and for all-cause mortality: multivariate Cox models.

	CHD + stroke mortality	p	All-cause mortality	p
Age (years)	1.17 (1.10-1.25)	<0.0001	1.17 (1.13-1.22)	<0.0001
Male gender	–		1.94 (1.31-2.87)	0.0009
Liver disease	5.23 (0.66-41.5)	0.11	2.24 (0.48-10.5)	0.27
AST (U/L)	–		1.02 (1.008-1.03)	0.0012
ALT (U/L)	–		0.99 (0.98-1.01)	0.58
γ-GT (U/L)	–		0.999 (0.997-1.00)	0.51
Diabetes	1.03 (0.38-2.77)	0.68	–	
Fasting glucose (mg/dL)	1.005 (0.991-1.019)	0.27	1.005 (0.997-1.013)	0.23
Insulin (mU/L)	1.026 (1.01-1.04)	0.0002	1.016 (1.006-1.03)	0.0015
Triglycerides (mg/dL)	1.003 (0.997-1.009)	0.38		
Coffe intake	0.52 (0.25-1.08)	0.11	0.78 (0.50-1.21)	0.22
Lp(a) ≥30 mg/L	1.45 (0.58-3.63)	0.43	0.84 (0.48-1.46)	0.77
Fibrinogen in 5th quintile	1.21 (0.43-3.35)	0.99	0.83 (0.47-1.47)	0.96
Lp(a) ≥30 mg/L and fibrinogen in 5th quintile	3.11 (1.26-7.65)	0.014	1.53 (0.76-3.06)	0.22

The models include variables with p values <0.1 at univariate analysis. Hazard ratios for continuous variables are reported for unitary increase.

(HR=1.61; 95%CI: 0.79-3.28, p=0.19), and with fasting glucose, insulin and triglyceride levels (Table 3). Lp(a) levels ≥30 mg/dL and fibrinogen levels in the 5th quintile of the gender-specific distributions resulted in hazard ratios of 1.89 and 1.68 respectively (p≥0.08); however, the combination of Lp(a) levels ≥30 mg/dL and of fibrinogen levels within the 5th quintile of gender-specific distributions was significantly associated with combined CHD and stroke mortality (Table 3).

In multivariate, stepwise Cox models (Table 4), all-cause mortality was positively associated with age, male gender, AST and insulin levels. The combination of CHD and stroke mortality was associated with age, insulin levels and the combination of Lp(a) levels ≥30 mg/dL and fibrinogen levels in the 5th quintile of the gender-specific distributions (Table 4).

Kaplan-Meier estimates of survival from all-cause mortality and from CHD and stroke mortality are separately shown in Figure 2 for: a) subjects with Lp(a) levels <30 mg/dL and fibrinogen levels in the lower four quintiles of the gender-specific distributions, b) subjects with either Lp(a) levels ≥30 mg/dL or fibrinogen levels in the 5th quintile of the gender-specific distributions, c) subjects with both Lp(a) levels ≥30 mg/dL and fibrinogen levels in the 5th quintile of the gender-specific distributions.

CRP levels were determined using a high sensitivity technique in citrated plasma from 447 subjects (80.0%). CRP levels were similar in men and women (Table 1) and were not significantly associated with a history of ischemic heart, cerebrovascular or peripheral artery disease (p=0.5) or with blood pressure (p≥0.2), but they were higher in subjects with diabetes (1.73 mg/L and

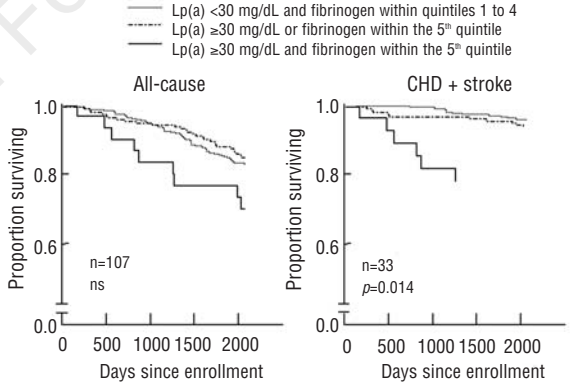

Figure 2. Kaplan Meier estimates of survival from all-cause mortality (left panel) and from CHD and stroke mortality (right panel) according to Lp(a) and fibrinogen levels. Survival curves are shown separately for subjects with Lp(a) levels <30 mg/dL and fibrinogen levels in the lower four quintiles of the gender-specific distributions (intermittent dotted line), subjects with either Lp(a) levels ≥30 mg/dL or fibrinogen levels in the 5th quintile of the gender-specific distributions (dotted line), and subjects with both Lp(a) levels ≥30 mg/dL and fibrinogen levels in the 5th quintile of the gender-specific distributions (continuous line).

43.15 mg/L, median and range, vs 1.23 mg/L and 29.15 mg/L, p=0.01) and in subjects with metabolic syndrome (1.80 mg/L and 43.15 mg/L vs 1.19 mg/L and 29.15 mg/L, p<0.0001), and were positively correlated with fibrinogen (r=0.38, p<0.0001) and insulin (r=0.23, p<0.0001), but not with Lp(a) levels (p=0.6). Twenty-five of these 447 subjects died of CHD or stroke during the follow-up. When restricting analysis to this group of

subjects, CRP levels were not predictive of combined CHD and stroke mortality (p=0.49, univariate analysis). In the same group of subjects the combination of high fibrinogen and Lp(a) predicted combined mortality (HR=4.57; 95%CI: 1.69-12.3, p=0.003) and the HR was virtually unchanged when forcing CRP levels into the multivariate Cox model (4.50;1.66-12.2, p=0.003).

Discussion

Several prospective studies have reported that raised levels of Lp(a) and fibrinogen are independent risk factors for cardiovascular morbidity and mortality.[5-7,16,17] High levels (in the highest tertile) of fibrinogen or of Lp(a) have been reported to increase the risk of fatal or non-fatal CHD by approximately 1.8-fold[6] and 1.7-fold.[7] The information relative to these markers as risk factors for ischemic stroke in prospective studies is more limited. High fibrinogen levels have been recognized as an independent risk factor for a first episode of ischemic stroke[17,18-21] and for stroke recurrence.[22] High Lp(a) levels have also been reported in association with cerebrovascular disease.[1,23-26] In a prospective study, elderly men, but not women, in the highest quintile of the Lp(a) distribution had a three times higher risk of stroke than those in the lowest quintile.[1] In another study, a one standard deviation increase in Lp(a) levels was associated with a 25% increased risk of ischemic stroke.[26]

That an interaction between fibrinogen and Lp(a) levels enhances the risk of myocardial infarction was first suggested in 1994,[27] but only few prospective studies have since evaluated such an interaction in the setting of incident CHD and related mortality. In men without clinical CHD, the combination of fibrinogen levels above the median value of the distribution and of Lp(a) levels ≥30 mg/dL increased the risk of CHD over a 5-year follow-up period by 2.5-fold.[28] In women enrolled in the Nurses' Health Study, the odds ratio for CHD events over 8 years of follow-up was 1.9 (95% C.I.: 1.3-3.0) for those with Lp(a) levels ≥30 mg/dL, compared to those with Lp(a) levels < 30 mg/dL.[29] Women with high levels of Lp(a) and with fibrinogen levels in the 5th quintile of the distribution had an odds ratio of 3.2 (95% CI: 1.6–6.5) for CHD, when compared with those who had low levels of both Lp(a) and fibrinogen (p interaction=0.05).[29] These studies did not take into account the contribution of hyper-insulinemia, an established independent risk factor for cardiovascular diseases[30] and cardiovascular mortality.[31]

In our study, we prospectively assessed the independent contribution of high fibrinogen and Lp(a) levels on incident CHD-and ischemic stroke-related mortality over a median follow-up of 6.3 years in an unselected population of women and men aged 65 years or more from a region in northern Italy. After adjustment for a number of demographic, clinical and laboratory characteristics, neither Lp(a) nor fibrinogen levels were significantly associated with the combined end-point of CHD- or ischemic stroke-related mortality. However, in addition to increasing age and hyper-insulinemia, the combination of Lp(a) levels ≥30 mg/dL and fibrinogen levels in the 5th quintile of the gender-specific distributions increased the risk of the incident end-point during follow-up by approximately 3-fold. Gender-related differences in fibrinogen, but not in Lp(a) metabolism have been reported previously,[32] and accordingly we found a difference in fibrinogen levels between men and women, also linked to alcohol ingestion[32] and liver function parameters. Although the risk contributed by increased Lp(a) and fibrinogen appeared greater in women than in men, mainly due to a higher rate of incident CHD-related mortality in women, this difference did not reach statistical significance.

Fibrinogen is an acute phase reactant and, rather than being a causal risk factor, may either be a marker of the atherosclerotic process[33] or reflect the low-grade inflammation associated with cardiovascular events.[34] The influence of fibrinogen levels on the incidence of myocardial infarction and death has been reported to be modified by other inflammation-sensitive proteins,[35] and in the study by Shai *et al.*[29] women with high levels of both Lp(a) and CRP (≥3 mg/L) had an odds ratio for CHD events similar to that of women with the association of high Lp(a) and fibrinogen levels. Insulin resistance has been associated with increased fibrinogen,[32,36] and insulin levels have been reported to be positively correlated with markers of inflammation.[37] CRP levels were measured in 80% of our population. These levels, which were similar in men and women, were higher in subjects with diabetes or with the metabolic syndrome and were positively correlated with fibrinogen and insulin, but not with Lp(a) levels. The association between moderately elevated CRP levels and an increased risk of developing cardiovascular disease has been consistently observed.[38] In our population, however, CRP levels were not predictive of combined CHD- and stroke-related mortality, and when this variable was forced into the multivariate Cox model, the hazard ratios for high fibrinogen and Lp(a) levels – and insulin levels (*not shown*) – remained virtually unchanged. In one prospective study conducted in men, CRP levels were significantly correlated with a number of variables, including fibrinogen and insulin levels, and the associations of CRP levels with incident ischemic heart disease and total mortality were completely abolished by controlling for fibrinogen.[39] In line with our findings, the power of fibrinogen to explain the association of CRP with incident ischemic heart disease – also observed by Thompson *et al.*[40] – suggests that fibrinogen could be a more specific risk factor than CRP. The meta-analysis of the Fibrinogen Studies Collaboration reported hazard

ratios for CHD and stroke close to 2.0 for a 1 g/L increase in fibrinogen levels,[17] and in a subset of 7011 participants with available CRP values, the findings for CHD were essentially unchanged following additional adjustment for CRP. While an active role for CRP in cardiovascular diseases has been questioned recently,[41] in transgenic mice with a 2-fold elevation in plasma fibrinogen levels, enhanced *in vivo* fibrin formation was associated with altered vascular remodeling.[42]

Important limitations of our study are represented by the relatively small size of the elderly population and the low number of fatal events due to CHD or stroke. We could not analyze differences in risk factors for the two categories of events, nor gender-related differences. Our findings do, however, provide a clinical corroboration of experimental data suggesting that an interaction between high fibrinogen and Lp(a) levels increases the risk of fatal vascular events in elderly subjects.

GM and FS were responsible for laboratory tests and for the database of the study. GR, PG and GC contributed to the design of the study and of the database. ADA and GC were responsible for the analysis of the results and the preparation of the manuscript. All the authros contributed to the ciritical evaluation of the final version of the paper. The authors declare that they have no potential confilct of interest. Funding: This study was supported in part by a grant from the Italian Ministry of Health (Ricerca Finalizzata 2003).

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