



# Increased homocysteine and lipoprotein(a) levels highlight systemic atherosclerotic burden in patients with a history of acute coronary syndromes

Gabriele Cioni, MD, PhD,<sup>a</sup> Rossella Marcucci, MD, PhD,<sup>a</sup> Anna Maria Gori, MSc,<sup>a</sup> Serafina Valente, MD,<sup>a</sup> Cristina Giglioli, MD,<sup>a</sup> Gian Franco Gensini, MD,<sup>a,b</sup> Rosanna Abbate, MD,<sup>a</sup> and Maria Boddi, MD, PhD,<sup>a</sup> *Florence, Italy*

**Background:** Strong evidence supports an association between high levels of homocysteine (Hcy) and lipoprotein(a) [Lp(a)] and an increased rate of ischemic vascular events.

**Methods:** The study population comprised 162 patients (50 women [30.9%]; age, 66.71 ± 12.76 years) having a history of acute coronary syndrome within 1 year who underwent fasting blood sampling, measurement of intima-media thickness and pulse wave velocity at the common carotid and femoral arteries by Doppler ultrasound, and ankle-brachial index measurement. Cutoff values were considered 0.9 mm and 1.2 mm for carotid and femoral intima-media thickness, respectively; 12 m/s for pulse wave velocity; and <0.9 for ankle-brachial index. We included hypertension, dyslipidemia, diabetes, overweight/obesity, smoking, and family history of cardiovascular disease in the count of traditional risk factors (CRFs). Adding Hcy ≥15 μmol/L and Lp(a) ≥500 mg/L to CRFs, we obtained a new score, named TOTAL.

**Results:** On univariate analysis, Hcy and Lp(a) were significantly associated with presence of atherosclerotic extracoronary lesions (for Hcy: β = .934; standard error = 0.178; *P* < .0001; for Lp(a): β = .961; standard error = 0.177; *P* < .0001) and compliance alterations (for Hcy: odds ratio, 13.3; 95% confidence interval, 3.9-45.3; *P* < .0001; for Lp(a): odds ratio, 14.6; 95% confidence interval, 5.69-37.62; *P* < .0001). On multivariate analysis, Lp(a) and Hcy were significantly associated with extracoronary atherosclerosis, even after correction for CRFs. The area under the curve of the TOTAL score for both atherosclerosis and vascular compliance alterations was significantly higher than the area under the curve of traditional CRFs plus only Hcy ≥15 μmol/L or plus Lp(a) ≥500 mg/L, separately added.

**Conclusions:** The addition of evaluation of Hcy ≥15 μmol/L and Lp(a) ≥500 mg/L to the traditional CRF count does improve detection of systemic atherosclerotic burden of patients with acute coronary syndrome and can offer a new opportunity to optimize secondary prevention. (*J Vasc Surg* 2016;64:163-70.)

Recent evidence has shown that several plasma biomarkers are higher in coronary artery disease (CAD) patients with concomitant peripheral arterial disease (PAD) than in patients with exclusive CAD.<sup>1</sup> A role for homocysteine (Hcy) and lipoprotein(a) [Lp(a)] has been hypothesized but not clearly proved.<sup>2</sup> Specifically, hyperhomocysteinemia is an independent risk factor for both PAD and CAD,<sup>3-5</sup> and in secondary prevention, elevated levels of Hcy were related to residual cardiovascular (CV) risk in patients with acute coronary syndrome (ACS) independently of the efficacy of statin treatment.<sup>6</sup>

Lp(a) levels are positively associated with CAD events, and the Copenhagen City Heart Study showed a stepwise increase in the risk of CAD associated with the increase

in Lp(a) levels without evidence of a threshold effect. Only one population-based study reported a significant association of Lp(a) and the risk of PAD.<sup>7</sup> The combination of high Hcy and Lp(a) levels synergistically increases the likelihood for development of CAD<sup>8</sup> and may significantly influence clinical outcome. No data are available about the association of high Hcy and Lp(a) levels with extracoronary atherosclerotic burden in ACS patients.

To test the hypothesis that high Hcy and Lp(a) levels could be related to the severity of asymptomatic peripheral atherosclerotic burden in ACS patients besides the occurrence of the traditional CV risk profile and the severity of CAD, we evaluated subclinical extracoronary atherosclerotic morphologic and functional damage by ultrasound examination<sup>9-15</sup> and the assay of Hcy and Lp(a) levels in 162 patients who in the last year had suffered a first ACS event, asymptomatic for extracoronary atherosclerosis involvement.

## METHODS

**Study population.** The study population consisted of 162 patients (50 women [30.9%]; age, 66.71 ± 12.76 years) having a history of ACS within 1 year, including ST-segment elevation myocardial infarction, non-ST-segment elevation myocardial infarction, and unstable angina,<sup>16</sup> asymptomatic for extracoronary (carotid, inferior limb, renal, and aortoiliac

From the Department of Experimental and Clinical Medicine, University of Florence, AOU-Careggi<sup>a</sup>; and the Don Carlo Gnocchi Foundation, IRCCS.<sup>b</sup>

Author conflict of interest: none.

Correspondence: Gabriele Cioni, MD, PhD, Department of Experimental and Clinical Medicine, University of Florence, Largo Brambilla 3, 50132 Florence, Italy (e-mail: [gabriele.cioni@unifi.it](mailto:gabriele.cioni@unifi.it)).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

0741-5214

Copyright © 2016 by the Society for Vascular Surgery. Published by Elsevier Inc.

<http://dx.doi.org/10.1016/j.jvs.2016.01.056>

arteries) atherosclerotic involvement, who had been enrolled from July 2009 to December 2011 on an outpatient basis at the Office for Vascular Function Assessment, Department of Experimental and Clinical Medicine, Careggi Hospital, Florence. All subjects gave written informed consent to the collection and analysis of data. The investigation was performed in accordance with the Declaration of Helsinki. Clinical practice, presented as the object of this study, represents the standard practice of our center and did not require Institutional Review Board approval.

The presence of cardiovascular risk factors (CRFs) was assessed according to the current guidelines: male sex; hypertension (systolic blood pressure [SBP]  $\geq 140$  mm Hg or diastolic blood pressure [DBP]  $\geq 90$  mm Hg, according to the guidelines of the European Society of Hypertension and the European Society of Cardiology, or taking an antihypertensive treatment<sup>17</sup>); hyperlipidemia (total serum cholesterol level  $\geq 160$  mg/dL, according to the Third Report of the National Cholesterol Education Program and European Atherosclerosis Society 2012 guidelines, or taking lipid-lowering medication); diabetes mellitus (treated with an oral hypoglycemic agent, insulin, or both or having fasting glucose levels  $\geq 126$  mg/dL, in agreement with the American Diabetes Association); family history of cardiovascular disease (CVD) (having first- or second-degree relatives with premature CV disease); and smoking habit, considered as present smoking habit or past from a period of  $< 1$  year.

According to the blood pressure values reported by the guidelines for the management of high blood pressure published in 2014, blood pressure control was considered good when values  $\leq 149/90$  mm Hg were measured at all control visits.<sup>17</sup>

According to the World Health Organization indications, a glycated hemoglobin (HbA<sub>1c</sub>) value of 6.5% has been considered good.<sup>18</sup>

**Study design.** Patients were evaluated 1 year after the ACS event. The follow-up was programmed within a maximum interval of 15 days from the programmed data. Clinical evaluation was performed in a quiet room and consisted of a dedicated medical questionnaire; physical examination with heart rate, arterial blood pressure, and body mass index (BMI) measurement; and blood sampling.

After the patient rested for at least 15 minutes in the supine position, a single operator performed electrocardiographic and ultrasound assessment. Exclusion criteria comprised autoimmune, pulmonary, or hematologic disorders; anxiety or depression; and history of alcoholism or addiction.

**Traditional CRFs and TOTAL CV score.** We considered traditional CRFs to be male sex; family history of CVD; dyslipidemia or low-density lipoprotein cholesterol (LDL-C) level  $\geq 70$  mg/dL; hypertension or SBP  $\geq 140$  mm Hg and DBP  $\geq 90$  mm Hg for nondiabetics and SBP  $\geq 120$  mm Hg and DBP  $\geq 80$  mm Hg for diabetics; BMI  $\geq 25$ ; diabetes; and smoking habit.

For each patient, we calculated a CV risk score, which we named the TOTAL score, by the addition of 1 point for Hcy  $\geq 15$   $\mu$ mol/L and Lp(a)  $\geq 500$  mg/L to the points due to the presence of traditional CRFs (male sex, family history of CVD, BMI  $\geq 25$ , diabetes, not good blood pressure control, LDL-C  $> 70$  mg/dL, or smoking habit). Minimum and maximum score values are 0 and 9, respectively.

**Ultrasound assessment.** All patients underwent ultrasound assessment by a MyLab 70 XVision machine equipped with a 7.5 MHz linear probe (Esaote Medical Systems, Rome, Italy). The system used dedicated software for computerized measurement of intima-media thickness (IMT) and arterial wall compliance (RF-data technology involving RF Quality Intima-Media Thickness [<sup>RF</sup>QIMT] and RF Quality Arterial Stiffness [<sup>RF</sup>QAS], Esaote Medical Systems).

IMT of the right and left common carotid arteries was measured in the 1-cm segment proximal to the carotid dilation with B-mode ultrasonography, by computerized probe using a 7.5 MHz transducer attached to a MyLab 70 XVision Esaote machine. Femoral IMT (f-IMT) was measured in the far wall of a 1-cm-long arterial segment proximal to the femoral bifurcation.

Arterial stiffness was expressed as local pulse wave velocity (PWv) and the change in distensibility of the right and left common carotid arteries and was measured in the 1-cm segment proximal to the carotid dilation. Femoral PWv (f-PWv) was measured in the far wall of a 1-cm-long arterial segment proximal to the femoral bifurcation. The cutoff value for PWv at the common carotids and femoral arteries was considered 12 m/s, according to current literature.<sup>17</sup>

For each subject, the maximum carotid IMT (c-IMT) or f-IMT and PWv values were used for statistical analysis. According to the guidelines of the European Society of Hypertension, c-IMT values  $\geq 0.9$  mm and f-IMT values  $\geq 1.2$  mm were considered abnormal.<sup>17</sup>

The ankle-brachial index (ABI) was determined by ultrasonographic method using a MyLab 70 XVision machine equipped with a 7.5 MHz linear probe and an aneroid sphygmomanometer with cuff inflators appropriate for brachial circumference. SBP measurements of the brachial, tibial posterior, and dorsalis pedis arteries were taken on both sides of the limb. The ABI was calculated as the ratio between the lowest ankle systolic pressure and the highest brachial systolic pressure.<sup>17,19,20</sup> ABI was determined for each leg, and the lowest value was considered for analysis. ABI values  $< 0.9$  and  $> 1.3$  were considered abnormal.<sup>6,21,22</sup>

We called atherosclerotic lesions the presence of at least one alteration of c-IMT or f-IMT, according to a previously defined cutoff.

**Coronary artery atherosclerosis.** Coronary angiography was performed only at the acute event, 1 year before the follow-up of this study, and it was not repeated at the control visit. Data of coronary angiography were collected from the medical history of every patient. The examination

was performed by standard techniques. The severity of coronary lesions was determined by visual estimation or by a quantitative coronary angiography program (Xcelera; Philips Healthcare, Best, The Netherlands) at the discretion of the interventional cardiologist performing the procedure. Clinically significant CAD was defined as the presence of a coronary lesion resulting in a lumen diameter stenosis >75% in a major epicardial artery (left anterior descending artery, left circumflex artery, right coronary artery) or one of its major branches. Clinically significant left main disease was defined as a lumen diameter stenosis >50%.<sup>13</sup>

Patients were stratified according to the number of involved vessels as follows: normal coronaries or nonobstructive CAD (subjects not showing the criteria for clinically significant CAD); one-vessel, two-vessel, and three-vessel disease (subjects showing significant lesions in one, two, and three vessels, respectively); and significant disease of the left main coronary artery, independent of concomitant lesions in other vessels.<sup>23</sup>

**Blood collection.** Venous blood samples were taken from each patient in the morning, after an overnight fast, and collected from the antecubital vein into evacuated plastic tubes (Vacutainer).

**Biohumoral parameters.** Lp(a) was measured by an immunonephelometric method (LPAX IMAGE; Beckman Coulter, Brea, Calif); values  $\geq 500$  mg/L were considered abnormal. Hcy plasma values (free or linked to proteins) were measured by immunoenzymatic assay (fluorescence polarization immunoassay, IMx; Abbott Laboratories, Oslo, Norway); values  $\geq 15$   $\mu\text{mol/L}$  were considered abnormal. Lipid profile was assessed by standard method.

**Statistical analysis.** Database construction and statistical analysis were performed with SPSS for Windows (version 19; SPSS Inc, Chicago, Ill). Categorical variables were expressed as frequencies and percentages; analysis of data distribution was evaluated by  $\chi^2$  test (statistical significance was for  $P < .05$ ). Continuous variables were expressed as mean value and standard deviation and median and range when appropriate.

According to the literature, normal values of ABI, IMT, and PWv were considered, respectively,  $\geq 0.9$ ,  $< 0.9$  for c-IMT and  $< 1.2$  for f-IMT, and  $< 12$  m/s for both carotid PWv (c-PWv) and f-PWv.

The nonparametric Mann-Whitney and Kruskal-Wallis tests were used for analysis of unpaired data. Correlation analysis was measured by using the Spearman correlation test.

Univariate and multivariate logistic regression tests were used to evaluate the association between independent risk factors and vascular dysfunction. Regression coefficients and 95% confidence intervals (CIs) are presented.

A  $P$  value  $< .05$  was considered to indicate statistical significance. We used receiver operating characteristic (ROC) curve analysis to evaluate the accuracy in discriminating the presence of peripheral atherosclerosis or altered vascular compliance in the study population.

ROC curves were performed using, as continuous variable, the probability of the number of traditional CRFs and the number of traditional CRFs plus Hcy  $\geq 15$   $\mu\text{mol/L}$  or Lp(a)  $\geq 500$  mg/L, or both, related to the presence of atherosclerosis or altered vascular compliance in the study population.

## RESULTS

**Demographic and clinical characteristics of the study population.** Clinical and biochemical features of the study population are listed in Table I.

Of 162 patients, 129 (79.6%) showed at least one of the traditional CRFs that did not match the target values indicated by the European Society of Cardiology and the European Atherosclerosis Society 2012 guidelines for the optimization of CV management in high-risk patients (Table I). One year after the ACS event, 74% of patients had not stopped smoking and about 50% showed BMI  $> 25$ , LDL  $> 70$  mg/dL, and not good HbA<sub>1c</sub> control.

All patients showed liver and renal function within normal limits.

Nobody reported a new acute coronary event after discharge from the coronary intensive care unit.

**Coronary atherosclerosis.** Regarding coronary artery involvement, we compared data of coronary angiograms, obtained at the acute cardiac event, with extracoronary data and biochemical parameters, collected after 1 year. In particular, 19 (11.5%) were one-vessel, 63 (39.1%) were two-vessel, and the remaining 80 (49.4%) were three-vessel CAD patients.

In our patients, the presence of traditional CRFs was not associated with coronary lesion extension, with the exception for smoking habit (odds ratio [OR], 1.9; 95% CI, 1.02-4.4;  $P = .046$ ) and diabetes (OR, 7.2; 95% CI, 2.7-18.3;  $P = .044$ ).

We found an association between severity of CAD and extracoronary atherosclerotic markers, such as ABI values (one-vessel,  $0.99 \pm 0.13$ ; two-vessel,  $0.89 \pm 0.20$ ; three-vessel,  $0.79 \pm 0.13$ ;  $P$  for trend = .042), c-IMT ( $1358 \pm 1019$  mm,  $1750 \pm 840$  mm, and  $2625 \pm 1117$  mm, respectively;  $P$  for trend = .039), and f-IMT ( $1552 \pm 985$  mm,  $1995 \pm 978$  mm, and  $2710 \pm 615$  mm;  $P$  for trend = .047).

Lp(a) and Hcy levels progressively increased according to CAD severity. One-vessel CAD patients showed lower Hcy ( $5.2 \pm 2.5$  mmol/L) and Lp(a) ( $157.7 \pm 347.9$  mg/L) values than two-vessel (Hcy,  $13.2 \pm 4.9$  mmol/L; Lp(a),  $579.7 \pm 517.9$  mg/L;  $P = .001$ ) and three-vessel CAD patients (Hcy,  $16.9 \pm 5.8$ ; Lp(a),  $845.2 \pm 474.5$ ;  $P < .0001$ ).

On univariate logistic regression analyses, we found a significant association between CAD severity and values of Lp(a)  $\geq 500$  mg/L (OR, 7.2; 95% CI, 2.7-18.3;  $P < .0001$ ) and Hcy  $\geq 15$   $\mu\text{mol/L}$  (OR, 12.8; 95% CI, 4.8-34.1;  $P < .0001$ ). These associations remained significant after adjustment for sex, age, overweight, nonoptimal LDL and high-density lipoprotein values, diabetes,

**Table I.** Study population characteristics at the visit time

|   |                        |
|---|------------------------|
| Clinical characteristics                                  |                        |
| Male  | 112 (69.1)             |
| Age, years  | 66.71 ± 12.76          |
| Smoking cessation <1 year                                 | 10 (26.3) <sup>a</sup> |
| Smoking habit <sup>b</sup>                                | 28 (17.3)              |
| SBP, mm Hg  | 127.28 ± 18.02         |
| DBP, mm Hg  | 76.57 ± 7.50           |
| BP values >140/90 mm Hg <sup>b</sup>                      | 42 (26.0)              |
| BMI, kg/m <sup>2</sup>                                    | 26.25 ± 3.67           |
| BMI ≥25 kg/m <sup>2b</sup>                                | 74 (45.67)             |
| Diabetes <sup>b</sup>                                     | 34 (21.0)              |
| Hypertension  | 97 (60.0)              |
| Dyslipidemia  | 86 (53.1)              |
| PAD   | 24 (14.8)              |
| Family history of CAD                                     | 54 (33)                |
| Pharmacologic therapies                                   |                        |
| Antihypertensive therapy                                  | 162 (100)              |
| ACE inhibitors  | 52 (32.1)              |
| ATII blockers   | 30 (18.5)              |
| Beta-blockers   | 61 (37.7)              |
| Calcium antagonists                                       | 15 (9.3)               |
| Diuretics   | 31 (19.1)              |
| Statins   | 162 (100)              |
| Antiplatelets   | 162 (100)              |
| OAT   | 15 (9.3)               |
| Oral hypoglycemic agents                                  | 34 (21.0)              |
| Biochemical parameters                                    |                        |
| LDL-C, mg/dL  | 98.11 ± 34.33          |
| LDL-C ≥70 mg/dL <sup>b</sup>                              | 86 (53.1)              |
| HDL-C, mg/dL  | 47.08 ± 16.97          |
| HDL-C ≤45 mg/dL <sup>b</sup>                              | 77 (47.5)              |
| Glycemia, nondiabetics, g/L                               | 0.97 ± 0.23            |
| Glycemia, diabetics, g/L                                  | 1.47 ± 0.58            |
| Diabetics with not optimal HbA <sub>1c</sub> <sup>c</sup> | 16 (47.1)              |
| Triglycerides, mg/dL                                      | 126 ± 48               |
| RBCs × 10 <sup>12</sup> /L                                | 4.47 ± 1.04            |
| WBCs × 10 <sup>9</sup> /L                                 | 5.76 ± 1.65            |
| Platelets × 10 <sup>9</sup> /L                            | 238 ± 121              |
| Hb, g/dL  | 13.8 ± 2.4             |
| Hct, %  | 38.9 ± 6.2             |
| Creatinine, mg/dL   | 0.9 ± 0.45             |
| AST, U/L  | 20 ± 14                |
| ALT, U/L  | 36 ± 11                |
| GGT, U/L  | 17 ± 12                |
| CK, U/L   | 201 ± 152              |
| Hcy, μmol/L   | 12.43 ± 6.00           |
| Hcy >15 μmol/L  | 58 (35.8)              |
| Lp(a), mg/L   | 512.31 ± 531.53        |
| Lp(a) >500 mg/L   | 83 (51.2)              |

ACE, Angiotensin-converting enzyme; ALT, alanine transaminase; AST, aspartate transaminase; ATII, angiotensin II; BMI, body mass index; BP, blood pressure; CAD, coronary artery disease; CK, creatine kinase; DBP, diastolic blood pressure; GGT, γ-glutamyltransferase; Hb, hemoglobin; HbA<sub>1c</sub>, glycated hemoglobin; Hct, hematocrit; Hcy, homocysteine; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); OAT, oral antiplatelet therapy; PAD, peripheral arterial disease; RBCs, red blood cells; SBP, systolic blood pressure; WBCs, white blood cells.

Categorical variables are presented as number (%). Continuous variables are presented as mean ± standard deviation.

<sup>a</sup>Percentage of total smokers at the event (n = 38).

<sup>b</sup>Traditional cardiovascular risk factors (CRFs) according to guidelines.

<sup>c</sup>Percentage of diabetics (n = 34).

arterial pressure not well controlled, overweight, and smoking habit for Lp(a) (OR, 3.8; 95% CI, 1.3-11.8; *P* = .018) and Hcy (OR, 4.9; 95% CI, 1.6-15.8; *P* = .007).

**Markers of extracoronary atherosclerosis and vascular compliance.** Table II shows the pattern of ultrasound morphologic and functional markers of extracoronary atherosclerotic vessel damage in ACS patients.

Of 162 patients, 45 (27.7%) did not show carotid or femoral atherosclerotic lesions (Table II). Age was significantly related to ABI (*r* = -0.272; *P* = .001), c-IMT (*r* = 0.292; *P* < .0001), and c-PWv (*r* = 0.284; *P* = .001) pattern. No differences according to gender or diabetes diagnosis were found (Table III).

Patients with family history of CVD showed a marked carotid and femoral involvement with respect to patients without family history (c-IMT, 1.9 ± 1.1 mm vs 1.3 ± 0.9 mm [*P* < .0001]; f-IMT, 2.1 ± 1.2 mm vs 1.3 ± 0.9 mm [*P* = .001], respectively) and the presence of ABI <0.9 (*P* < .0001).

Patients with severe CAD, defined as three-vessel impairment, had significantly higher prevalence of extracoronary atherosclerotic lesion involvement (72/80 [90%] vs 40/82 [52.5%]; *P* < .0001) than patients with one- or two-vessel CAD. On the univariate logistic analyses, we found that severe CAD was associated with extracoronary atherosclerosis (OR, 16.9; 95% CI, 2.2-131.4; *P* = .007). After adjustment for CRFs, this association remained significant (OR, 19.1; 95% CI, 2.2-164.3; *P* = .007).

**Hcy, Lp(a), and vascular impairment.** The relationships between Hcy and Lp(a) values and morphologic and functional markers of atherosclerotic damage and ABI are shown in Table II.

On the univariate logistic analyses, Hcy ≥15 μmol/L and Lp(a) ≥500 mg/L were significantly associated with CAD severity (Hcy: OR, 12.8; 95% CI, 4.8-34.1; *P* < .0001; Lp(a): OR, 7.2; 95% CI, 2.9-18.3; *P* < .0001), and these associations remained significant also after the adjustment for CRFs for Hcy ≥15 μmol/L (OR, 5.2; 95% CI, 4.8-34.1; *P* = .005).

IMT and PWv were significantly higher in patients with Hcy ≥15 μmol/L and Lp(a) ≥500 mg/L than in patients with normal Lp(a) and Hcy values (data not shown).

On univariate logistic analysis, Hcy ≥15 μmol/L and Lp(a) ≥500 mg/L were significantly associated with the presence of atherosclerotic extracoronary lesions (Hcy: OR, 13.3; 95% CI, 3.9-45.3; *P* < .0001; Lp(a): OR, 14.6; 95% CI, 5.69-37.62; *P* < .0001) and compliance alterations (Hcy: OR, 13.9; 95% CI, 6.2-31.2; *P* < .0001; Lp(a): OR, 2.7; 95% CI, 1.4-5.1; *P* = .003).

ROC curve analysis for TOTAL score showed a significantly higher area under the curve for the discrimination of atherosclerosis (abnormal on at least one-side for c-IMT, f-IMT, or ABI) and vascular compliance alterations (c-PWv or f-PWv) in the study population in comparison with only traditional CRFs (Table IV). Differences among ROC curves are expressed in Table IV.

## DISCUSSION

The novel finding of our study is that the contemporaneous occurrence of high Lp(a) and Hcy levels was strictly

**Table II.** Instrumental markers expressed in the total population and according to the number of risk factors, homocysteine (Hcy) and lipoprotein(a) [Lp(a)] levels, and TOTAL score

|                      | ABI              | c-IMT, mm        | f-IMT, mm        | c-PWv, m/s       | f-PWv, m/s       |
|----------------------|------------------|------------------|------------------|------------------|------------------|
| Total population     |                  |                  |                  |                  |                  |
| Variable             | 0.93 ± 0.18      | 1.6 ± 1.1        | 1.7 ± 1.1        | 10.37 ± 3.76     | 9.97 ± 3.88      |
| Impaired marker      | 70 (43.2)        | 91 (56.2)        | 82 (50.6)        | 49 (30.2)        | 58 (35.8)        |
| Traditional CRFs     |                  |                  |                  |                  |                  |
| ≤2 (n = 58)          | 1.01 ± 0.18      | 1529.78 ± 1013   | 1456.29 ± 1060   | 8.77 ± 2.58      | 8.95 ± 2.89      |
| 3-4 (n = 73)         | 0.90 ± 0.17      | 1639.21 ± 1018   | 1716.59 ± 1137   | 10.28 ± 4.23     | 10.42 ± 3.97     |
| >4 (n = 31)          | 0.82 ± 0.14      | 1868.27 ± 1083   | 1956.67 ± 919    | 11.92 ± 3.68     | 10.63 ± 4.97     |
| P for trend value    | <b>.001</b>      | <b>.09</b>       | <b>.024</b>      | <b>.069</b>      | <b>.26</b>       |
| Hcy                  |                  |                  |                  |                  |                  |
| >15 μmol/L (n = 60)  | 0.83 ± 0.15      | 1873.6 ± 1023.2  | 2274.1 ± 1289.8  | 12.8 ± 3.96      | 12.24 ± 4.2      |
| <15 μmol/L (n = 102) | 0.98 ± 0.16      | 1427.1 ± 1028.6  | 1340.3 ± 883.02  | 8.8 ± 2.74       | 8.7 ± 3.1        |
| P value              | <b>&lt;.0001</b> | <b>.001</b>      | <b>&lt;.0001</b> | <b>&lt;.0001</b> | <b>&lt;.0001</b> |
| Lp(a)                |                  |                  |                  |                  |                  |
| >500 mg/L (n = 87)   | 0.87 ± 0.15      | 2095.7 ± 1056.9  | 2074.5 ± 1128.1  | 11.05 ± 4.05     | 10.98 ± 3.71     |
| <500 mg/L (n = 75)   | 0.99 ± 0.17      | 1201.6 ± 901.7   | 1212.9 ± 1044.7  | 9.17 ± 2.99      | 9.23 ± 3.48      |
| P value              | <b>.001</b>      | <b>&lt;.0001</b> | <b>&lt;.0001</b> | <b>.001</b>      | <b>&lt;.05</b>   |
| TOTAL score          |                  |                  |                  |                  |                  |
| ≤2 (n = 45)          | 1.05 ± 0.31      | 1373.68 ± 1012   | 1279.74 ± 526    | 8.98 ± 2.77      | 8.72 ± 2.65      |
| 3-4 (n = 60)         | 0.95 ± 0.19      | 1622.29 ± 979    | 1799.21 ± 1123   | 9.93 ± 3.45      | 9.10.36 ± 3.72   |
| >4 (n = 57)          | 0.82 ± 0.15      | 1859.75 ± 1212   | 1980.60 ± 1113   | 12.71 ± 4.90     | 12.43 ± 4.29     |
| P for trend value    | <b>&lt;.0001</b> | <b>.004</b>      | <b>&lt;.0001</b> | <b>&lt;.0001</b> | <b>.003</b>      |

ABI, Ankle-brachial index; CRFs, cardiovascular risk factors; c-IMT, carotid intima-media thickness; c-PWv, carotid pulse wave velocity; f-IMT, femoral artery intima-media thickness; f-PWv, femoral artery pulse wave velocity. Categorical variables are presented as number (%). Continuous variables are presented as mean ± standard deviation. Bold values show statistically significant P values.

**Table III.** Extracoronary markers of atherosclerosis according to gender and diabetes

|                               | ABI         | c-IMT, mm  | f-IMT, mm  | c-PWv, m/s   | f-PWv, m/s   |
|-------------------------------|-------------|------------|------------|--------------|--------------|
| Men (n = 112; 69.1%)          |             |            |            |              |              |
| Variable                      | 0.96 ± 0.24 | 1.62 ± 1.3 | 1.70 ± 1.1 | 10.42 ± 3.93 | 10.11 ± 4.03 |
| Women (n = 50; 30.9%)         |             |            |            |              |              |
| Variable                      | 0.92 ± 0.17 | 1.61 ± 0.8 | 1.68 ± 1.1 | 10.21 ± 2.89 | 9.94 ± 3.84  |
| Diabetics (n = 34; 21.0%)     |             |            |            |              |              |
| Variable                      | 0.90 ± 0.27 | 1.73 ± 1.6 | 1.75 ± 1.3 | 11.07 ± 3.55 | 10.33 ± 3.97 |
| Nondiabetics (n = 148; 79.0%) |             |            |            |              |              |
| Variable                      | 0.93 ± 0.16 | 1.67 ± 1.4 | 1.77 ± 1.7 | 10.26 ± 3.49 | 9.91 ± 3.72  |

ABI, Ankle-brachial index; c-IMT, carotid intima-media thickness; c-PWv, carotid pulse wave velocity; f-IMT, femoral artery intima-media thickness; f-PWv, femoral artery pulse wave velocity. Values are reported as mean ± standard deviation. P values were not significant.

associated with the presence and severity of asymptomatic systemic atherosclerotic damage when added to the pattern of traditional CRFs (TOTAL score) and to CAD severity at the acute event.

A high percentage of our ACS patients showed a scarce control of traditional CRFs because of ongoing smoking, BMI >25, poor control of hypertension, and glycemic profile. Because the strongest CRFs are hypertension for cerebrovascular disease and smoking and diabetes for PAD, the demonstration of asymptomatic carotid and femoral vessel damage could help in selecting those ACS patients at high CV residual risk who especially need good control of CRFs.<sup>24</sup> Among investigated markers of systemic atherosclerotic damage, ABI <0.9 was the marker with

the highest prevalence in our ACS patients and could be proposed as the marker of choice for screening of asymptomatic peripheral atherosclerotic damage in this high-risk subpopulation.

In our ACS population, only a positive family history of CAD was associated with systemic atherosclerotic damage. Interestingly, the contemporaneous occurrence of high Lp(a) and Hcy levels added to the traditional CRF pattern (TOTAL score) highlighted the presence and severity of subclinical systemic atherosclerotic damage better than CAD severity. In our study, TOTAL score (traditional CRFs plus high Hcy and Lp(a) levels) was strongly linked to both morphologic and functional vessel damage due to the increase in Hcy and Lp(a) values according to the

**Table IV.** Differences among receiver operating characteristic (ROC) curves in relation to atherosclerosis in the study population

|                            | ROC curve, AUC (95% CI)       | AUC difference | 95% CI difference | P value |
|----------------------------|-------------------------------|----------------|-------------------|---------|
| <b>Atherosclerosis</b>     |                               |                |                   |         |
| CRFs vs                    | 0.65 (0.53-0.76) <sup>a</sup> | −0.05          | −0.08 to −0.03    | .0001   |
| CRFs + Hcy                 | 0.69 (0.59-0.78) <sup>b</sup> |                |                   |         |
| CRFs vs                    | 0.65 (0.53-0.76) <sup>a</sup> | −0.08          | −0.10 to −0.06    | <.0001  |
| CRFs + Lp(a)               | 0.69 (0.59-0.79) <sup>c</sup> |                |                   |         |
| CRFs vs                    | 0.65 (0.53-0.76) <sup>a</sup> | −0.12          | −0.15 to −0.09    | <.0001  |
| CRFs + Hcy + Lp(a)         | 0.73 (0.64-0.83) <sup>d</sup> |                |                   |         |
| CRFs + Hcy vs              | 0.68 (0.58-0.78) <sup>b</sup> | −0.05          | −0.07 to −0.03    | <.0001  |
| CRFs + Hcy + Lp(a)         | 0.73 (0.64-0.83) <sup>d</sup> |                |                   |         |
| CRFs + Lp(a) vs            | 0.69 (0.59-0.79) <sup>c</sup> | −0.05          | −0.05 to −0.04    | <.0001  |
| CRFs + Hcy + Lp(a)         | 0.73 (0.64-0.83) <sup>d</sup> |                |                   |         |
| <b>Vascular compliance</b> |                               |                |                   |         |
| CRFs vs                    | 0.64 (0.54-0.76) <sup>e</sup> | −0.10          | −0.11 to −0.08    | <.0001  |
| CRFs + Hcy                 | 0.72 (0.63-0.80) <sup>f</sup> |                |                   |         |
| CRFs vs                    | 0.64 (0.54-0.76) <sup>e</sup> | −0.03          | −0.06 to −0.01    | .0128   |
| CRFs + Lp(a)               | 0.65 (0.53-0.74) <sup>g</sup> |                |                   |         |
| CRFs vs                    | 0.64 (0.54-0.76) <sup>e</sup> | −0.11          | −0.15 to −0.07    | <.0001  |
| CRFs + Hcy + Lp(a)         | 0.73 (0.65-0.81) <sup>h</sup> |                |                   |         |

AUC, Area under the curve; CI, confidence interval; CRFs, cardiovascular risk factors; Hcy, homocysteine; Lp(a), lipoprotein(a).

<sup>a</sup>P value = .03.

<sup>b</sup>P value < .0001.

<sup>c</sup>P value = .0002.

<sup>d</sup>P value < .0001.

<sup>e</sup>P value = .02.

<sup>f</sup>P value < .0001.

<sup>g</sup>P value = .002.

<sup>h</sup>P value < .0001.

increase in IMT or vascular stiffness at the carotid and femoral arteries (Fig).

In primary CV prevention, the role of Hcy and Lp(a) as lipid and thrombotic biomarkers in the individual CV risk stratification was proposed but is still under debate; no data are available for the use of Hcy values in residual CV risk measurement in ACS patients, whereas a possible association between high Lp(a) levels and a persistent high CV risk in CV patients receiving statin therapy<sup>25,26</sup> was proposed.

The strict association between high Lp(a) and Hcy levels and positive family history of CV disease found in our ACS patients strongly supports the role of the genetic component in determining high levels of both Hcy and Lp(a). Our findings do not allow us to know what pathophysiologic mechanisms facilitating the occurrence and progression of atherosclerotic damage and recurrence of CV events could be associated with high Lp(a) and Hcy levels, but proatherogenic, prothrombotic, and antifibrinolytic activities were clearly reported. Indeed, an amplification of oxidative stress, promotion of endothelial cell necrosis and of monocyte and macrophage migration with foam cell formation, and expanding necrotic lipid core could all contribute to the damage of the CV system<sup>27</sup> associated with high Hcy and Lp(a) levels.

According to recent advances, our data may prove useful not only to optimize the stratification of patients

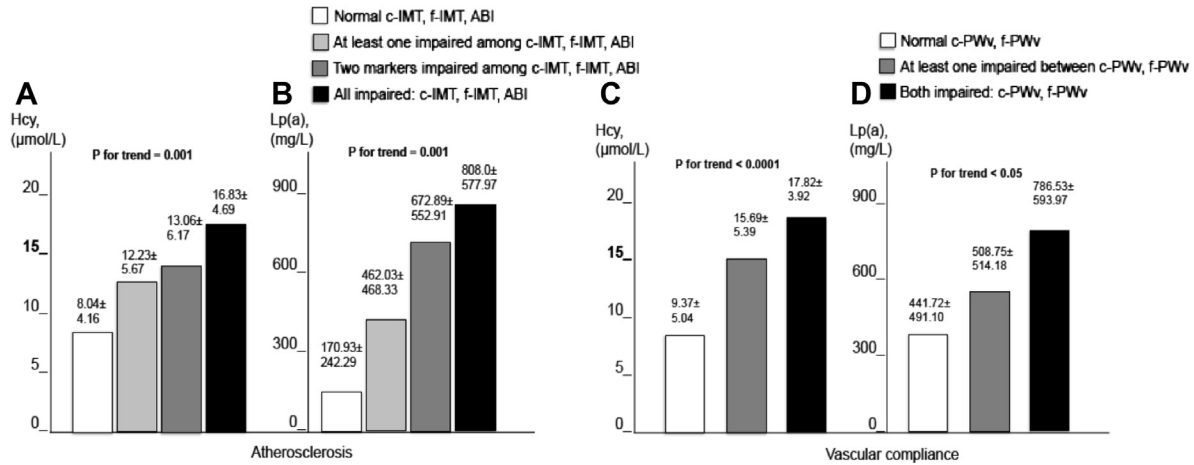
at increased risk of disseminated atherosclerosis but also to identify those subgroups for whom conventional therapies would not be able to correct the risk factors effectively. In particular, ongoing studies<sup>28,29</sup> showed that proprotein convertase subtilisin kexin 9 (PCSK9) monoclonal antibodies were able to reduce Lp(a) plasma levels. Regarding the use of vitamin supplementation to reduce CV risk, there are no convincing data that support a positive clinical effect by the reduction of Hcy levels.

Dedicated studies are needed to investigate whether high Lp(a) and Hcy levels that are associated with more severe systemic atherosclerotic damage could also select the ACS subpopulation with very high residual CV risk.

**Limits.** Our study is an observational investigation. A prospective phase has been started to investigate the possible clinical relevance of our findings in the calculation of residual CV risk in ACS patients with high TOTAL score and systemic atherosclerotic burden.

Among proposed lipid and thrombotic biomarkers of CV risk we studied, Lp(a) and Hcy levels are not markers of inflammation. It is possible that the addition of other markers of CV risk to the calculation of TOTAL score could improve the scoring of systemic atherosclerotic damage in ACS patients.

The TOTAL score is not actually validated in an independent population. A procedure of validation has been started.



**Fig. A and B,** Homocysteine (*Hcy*) and lipoprotein(a) [*Lp(a)*] values according to different degrees of extracoronary atherosclerosis. **C and D,** *Hcy* and *Lp(a)* values according to different degrees of vascular compliance. *ABI*, Ankle-brachial index; *c-IMT*, carotid intima-media thickness; *c-PWV*, carotid pulse wave velocity; *f-IMT*, femoral artery intima-media thickness; *f-PWV*, femoral artery pulse wave velocity.

### CONCLUSIONS

In our patients having a history of ACS within 1 year, the contemporaneous occurrence of high *Hcy* and *Lp(a)* levels added to the pattern of traditional CRFs (TOTAL score) was strictly related to the presence and severity of asymptomatic systemic morphologic and functional atherosclerotic damage that was detectable in about 50% of ACS patients.

### AUTHOR CONTRIBUTIONS

Conception and design: GC, RA, MB  
 Analysis and interpretation: GC, RA, MB  
 Data collection: GC, RM, SV, CG, RA, MB  
 Writing the article: GC, RA, MB  
 Critical revision of the article: GC, RM, RA, MB  
 Final approval of the article: GC, RM, AG, SV, CG, GG, RA, MB  
 Statistical analysis: GC, AG  
 Obtained funding: GG, RA, MB  
 Overall responsibility: RM

### REFERENCES

- Grenon SM, Vittinghoff E, Owens CD, Conte MS, Whooley M, Cohen BE. Peripheral artery disease and risk of cardiovascular events in patients with coronary artery disease: insights from the Heart and Soul Study. *Vasc Med* 2013;18:176-84.
- Mandelzweig L, Battler A, Boyko V, Bueno H, Danchin N, Filippatos G, et al; Euro Heart Survey Investigators. The second Euro Heart Survey on acute coronary syndromes: characteristics, treatment, and outcome of patients with ACS in Europe and the Mediterranean Basin in 2004. *Eur Heart J* 2006;27:2285-93.
- Khandanpour N, Loke YK, Meyer FJ, Jennings B, Armon MP. Homocysteine and peripheral arterial disease: systematic review and meta-analysis. *Eur J Vasc Endovasc Surg* 2009;38:316-22.
- Kumakura H, Kanai H, Araki Y, Hojo Y, Kasama S, Sumino H, et al. Differences in brain natriuretic peptide and other factors between Japanese peripheral arterial disease patients with critical limb ischemia

and intermittent claudication. *J Atheroscler Thromb* 2013;20:798-806.

- Martín-Herrero F, Martín-Moreiras J, Pabón P, Sánchez PL, Morfíño-Muñoz JL, Jimenez-Candil J, et al. Homocysteine and outcome in young patients with acute coronary syndromes. *Int J Cardiol* 2007;118:183-8.
- Peters SA, den Ruijter HM, Bots ML, Moons KG. Improvements in risk stratification for the occurrence of cardiovascular disease by imaging subclinical atherosclerosis: a systematic review. *Heart* 2012;98:177-84.
- Tsimikas S, Kiechl S, Willeit J, Mayr M, Miller ER, Kronenberg F, et al. Oxidized phospholipids predict the presence and progression of carotid and femoral atherosclerosis and symptomatic cardiovascular disease: five-year prospective results from the Bruneck study. *J Am Coll Cardiol* 2006;6:2219-28.
- Banos-Gonzalez MA, Angles-Cano E, Cardoso-Saldana G, Pena-Duque MA, Martinez-Rios MA, Valente-Acosta B, et al. Lipoprotein(a) and homocysteine potentiate the risk of coronary artery disease in male subjects. *Circ J* 2012;76:1953-7.
- Coskun U, Yildiz A, Esen OB, Baskurt M, Cakar MA, Kiklickesmez KO, et al. Relationship between carotid intima-media thickness and coronary angiographic findings: a prospective study. *Cardiovasc Ultrasound* 2009;7:59.
- Zielinski T, Dzielinska Z, Januszewicz A, Rynkun D, Makowiecka Ciesla M, Tyczynski P, et al. Carotid intima-media thickness as a marker of cardiovascular risk in hypertensive patients with coronary artery disease. *Am J Hypertens* 2007;20:1058-64.
- Lekakis JP, Papamichael CM, Cimponeriu AT, Stamatelopoulos KS, Papaioannou TG, Kanakakis J, et al. Atherosclerotic changes of extracoronary arteries are associated with the extent of coronary atherosclerosis. *Am J Cardiol* 2000;85:949-52.
- Sosnowski C, Pasiernski T, Janeczko-Sosnowska E, Szulczyk A, Dabrowski R, Woźniak J, et al. Femoral rather than carotid artery ultrasound imaging predicts extent and severity of coronary artery disease. *Kardiologia Pol* 2007;65:760-6; discussion: 767-8.
- Yang EY, Chambless L, Sharrett AR, Virani SS, Liu X, Tang Z, et al. Carotid arterial wall characteristics are associated with incident ischemic stroke but not coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) study. *Stroke* 2012;43:103-8.
- Koivisto T, Virtanen M, Hutri-Kähönen N, Lehtimäki T, Jula A, Juonala M, et al. Arterial pulse wave velocity in relation to carotid intima-media thickness, brachial flow-mediated dilation and carotid artery distensibility: the Cardiovascular Risk in Young Finns Study and the Health 2000 Survey. *Atherosclerosis* 2012;220:387-93.

15. Srámek A, Bosch JG, Reiber JH, Van Oostayen JA, Rosendaal FR. Ultrasound assessment of atherosclerotic vessel wall changes: reproducibility of intima-media thickness measurements in carotid and femoral arteries. *Invest Radiol* 2000;35:699-706.
16. Hamm CW, Bassand JP, Agewall S, Bax J, Boersma E, Bueno H, et al; European Society of Cardiology. ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2011;32:2999-3054.
17. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al; Task Force for the Management of Arterial Hypertension of the European Society of Hypertension and the European Society of Cardiology. 2013 ESH/ESC practice guidelines for the management of arterial hypertension. *Blood Press* 2014;23:3-16.
18. Use of glycosylated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. Geneva: World Health Organization; 2011. WHO/NMH/CHP/CPM/11.1.
19. Schröder F, Diehm N, Kareem S, Ames M, Pira A, Zwettler U, et al. A modified calculation of ankle-brachial pressure index is far more sensitive in the detection of peripheral arterial disease. *J Vasc Surg* 2006;44:531-6.
20. European Stroke Organisation, Tendera M, Aboyans V, Bartelink ML, Baumgartner I, Clément D, Collet JP, et al; ESC Committee for Practice Guidelines. ESC Guidelines on the diagnosis and treatment of peripheral artery diseases: document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteries: the Task Force on the Diagnosis and Treatment of Peripheral Artery Diseases of the European Society of Cardiology (ESC). *Eur Heart J* 2011;32:2851-906.
21. Allison MA, Aboyans V, Granston T, McDermott MM, Kamineni A, Ni H, et al. The relevance of different methods of calculating the ankle brachial index—the multi-ethnic study of atherosclerosis. *Am J Epidemiol* 2010;171:368-76.
22. Aboyans V, Criqui MH, Abraham P, Allison MA, Creager MA, Diehm C, et al. Measurement and interpretation of ankle-brachial index: a scientific statement from the American Heart Association. *Circulation* 2012;126:2890-909.
23. Patel MR, Dehmer GJ, Hirshfeld JW, Smith PK, Spertus JA. ACCF/SCAI/STS/AATS/AHA/ASNC 2009 appropriateness criteria for coronary revascularization: a report by the American College of Cardiology Foundation Appropriateness Criteria Task Force, Society for Cardiovascular Angiography and Interventions, Society of Thoracic Surgeons, American Association for Thoracic Surgery, American Heart Association, and the American Society of Nuclear Cardiology Endorsed by the American Society of Echocardiography, the Heart Failure Society of America, and the Society of Cardiovascular Computed Tomography. *J Am Coll Cardiol* 2009;53:530-53.
24. Erbel R, Budoff M. Improvement of cardiovascular risk prediction using coronary imaging: subclinical atherosclerosis: the memory of lifetime risk factor exposure. *Eur Heart J* 2012;33:1201-13.
25. Nordestgaard BG, Chapman MJ, Ray K, Borén J, Andreotti F, Watts GF, et al; European Atherosclerosis Society Consensus Panel. Lipoprotein(a) as a cardiovascular risk factor: current status. *Eur Heart J* 2010;31:2844-53.
26. Sillesen H, Muntendam P, Adourian A, Entreklin R, Garcia M, Falk E, et al. Carotid plaque burden as a measure of subclinical atherosclerosis: comparison with other tests for subclinical arterial disease in the High Risk Plaque BioImage Study. *JACC Cardiovasc Imaging* 2012;5:681-9.
27. Cai A, Li L, Zhang Y, Mo Y, Mai W, Zhou Y. Lipoprotein(a): a promising marker for residual cardiovascular risk assessment. *Dis Markers* 2013;35:551-9.
28. Desai NR, Kohli P, Giugliano RP, O'Donoghue ML, Somaratne R, Zhou J, et al. AMG145, a monoclonal antibody against proprotein convertase subtilisin kexin type 9, significantly reduces lipoprotein(a) in hypercholesterolemic patients receiving statin therapy: an analysis from the LDL-C Assessment with Proprotein Convertase Subtilisin Kexin Type 9 Monoclonal Antibody Inhibition Combined with Statin Therapy (LAPLACE)-Thrombolysis in Myocardial Infarction (TIMI) 57 trial. *Circulation* 2013;128:962-9.
29. Raal FJ, Giugliano RP, Sabatine MS, Koren MJ, Langslet G, Bays H, et al. Reduction in lipoprotein(a) with PCSK9 monoclonal antibody evolocumab (AMG 145): a pooled analysis of more than 1,300 patients in 4 phase II trials. *J Am Coll Cardiol* 2014;63:1278-88.

Submitted Jul 27, 2015; accepted Jan 31, 2016.