PAI-1 AND HOMOCYSTEINE, BUT NOT LIPOPROTEIN (a) AND THROMBOPHILIC POLYMORPHISMS, ARE INDEPENDENTLY ASSOCIATED WITH THE OCCURRENCE OF MAJOR ADVERSE CARDIAC EVENTS AFTER SUCCESSFUL CORONARY STENTING

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ABSTRACT

Objective: Scarce and contrasting data are available on the role of thrombophilic risk factors in the occurrence of major adverse cardiac events (MACE) after coronary stenting in ACS patients. Aim of our study was to evaluate the role of FV Leiden, prothrombin G20210A polymorphism, plasminogen activator inhibitor type-1 4G/5G polymorphism, plasminogen activator inhibitor type-1 (PAI-1), homocysteine (Hcy) and lipoprotein (a) [Lp(a)] in the occurrence of MACE in ACS patients who underwent coronary stenting.

Design: We enrolled 520 (375 M/145 F) ACS patients and 520 age and sex matched controls. MACE were recorded in 109 patients. Heterozygosity for FV Leiden, prothrombin G20210A polymorphism and 4G/5G polymorphism did not significantly differ between patients with and without MACE. A significantly higher percentage of patients with elevated Hcy and PAI-1 levels had MACE with respect to the others (28 vs 19%, p<.001 and 25% vs 15.7%, p<.001). At the Kaplan-Meier survival curves, the overall risk of MACE was significantly higher among patients with elevated PAI-1 and Hcy levels (p=.006 and .04). At Cox regression analysis - adjusted for age, gender, traditional cardiovascular risk factors, renal function, systolic left ventricular function, the number of stenosed vessels and history of PCI or CABG - Hcy and PAI-1 levels within the fifth quintile (with respect to the first) were significant and independent risk factors for the future occurrence of MACE (Hcy=OR 7.5 (95%CI 1.1-57.7), p<0.05; PAI-1=OR 5.3 (95%CI 1.2-23.8),p<0.05).

Conclusions: Our study demonstrates that elevated PAI-1 and Hcy levels are independent risk factors for MACE after successful coronary stenting, whereas Lp(a) and thrombophilic polymorphisms do not have any predictive role.

Keywords: coronary stenting, MACE, genetic polymorphisms, homocysteine, PAI-1.
INTRODUCTION
The introduction of intracoronary stents into clinical practice dramatically changed the strategy of treatment of coronary artery disease (CAD). In particular, in patients suffering from acute coronary syndromes (ACS), primary coronary angioplasty with stent implantation has been shown to reduce the in-hospital mortality rate [1][2]. As regards the incidence of major adverse cardiac events (MACE) – defined by death, myocardial infarction (MI) or target lesion revascularization (TLR) –, several clinical factors have been documented to be associated with an adverse outcome after ACS. In particular, the rapid increase in the number of stent implantations revealed the in-stent restenosis as a new entity with significant clinical and socio-economic implications. Currently, repeated angioplasty is the treatment of choice even if it is characterized by a 30% rate of MACE at 9 ± 4 months post the procedure. In 90% of cases, the rate of MACE is driven by the need for TLR, particularly as a result of recurrent restenosis.[2]

Ongoing secondary prevention efforts include the identification and validation of novel biochemical and genetic factors that increase the rate of MACE. Thrombophilic polymorphisms such as prothrombin gene mutation G20210A and factor V gene mutation G1619A - FV Leiden -, are established risk factors for venous thromboembolism [3], but their role in arterial thrombosis [4] is controversial and no data are available on their possible role in the occurrence of MACE after coronary stenting.

Data obtained on a limited number of patients are available on the association between plasminogen activator inhibitor 1 - PAI-1 - circulating levels and MACE after coronary stenting.[5][6][7][8] Contrasting data have been obtained on the association between homocysteine (Hcy) levels and both restenosis and mortality [9][10][11][12][13][14][15] in patients who underwent coronary revascularization with stent implantation whereas two studies on lipoprotein (a) [Lp(a)] levels and coronary stenting provided inconsistent results.[16][17]

Aim of our study was to thoroughly evaluate the association between the occurrence of MACE in patients with acute coronary syndrome (ACS) who underwent successful percutaneous coronary intervention (PCI) with stent implantation and the presence of genetic and metabolic thrombophilic risk factors – FV Leiden, prothrombin G20210A and PAI-1 4G/5G polymorphisms, PAI-1, Hcy and Lipoprotein (a) levels.

METHODS
Subjects Investigated
The study population included 530 consecutive adult patients admitted to the Coronary Care Unit of the University of Florence with diagnosis of acute myocardial infarction or unstable angina. All patients underwent coronary angiography performed by Judkins’ method and PCI with bar-metal stent implantation for de novo stenosis in ≥1 native coronary artery. Ten patients were excluded as PCI was not successful (>30% residual stenosis), so that the final patient group was formed by 520 patients. The diagnosis of unstable angina at presentation was based on a history of crescendo angina, or angina of new onset (within 1 month) in the absence of electrocardiographic and cardiac enzyme changes indicative of an acute MI. Acute MI was diagnosed as an increase in creatine kinase (or its MB isoenzyme) levels at least twice the upper normal limits with at least one of the following: acute onset of prolonged typical ischemic chest pain; ST-segment elevation or depression in ≥2 leads, ≥0.2 mV in V1-V3, >0.1 mV in other leads.

Blood samples were obtained at hospital admission.

The subjects were considered to have hypertension if they had been diagnosed as hypertensives according to the guidelines of European Society of Hypertension/European Society of Cardiology [18] or were taking antihypertensive drugs. A positive family history was defined as the presence of at least one first-degree relative who had developed CAD before the age of 55 years for men and 65 years for women. Dyslipidemia was defined
The control group consisted of 520 subjects, gender and age (by 10 yrs)- matched, recruited from the staff of the University of Florence and Careggi Hospital and from partners or friends of patients. A structured questionnaire to identify symptom-free controls and to exclude subjects who were suspected of having any form of vascular disease was used. Informed written consent was obtained from all subjects and the study was approved by the local Ethical Review Board.

**Follow-up**

Data were obtained by a structured telephone interview and a clinical evaluation in case of clinical recurrences and/or new PCI.

The clinical end-points were a composite of MACE including cardiac death (defined as death from MI, pump failure, sudden cardiac death or death because of arrhythmias), MI and TLR for symptomatic restenosis. Restenosis was defined as a diameter stenosis $\geq$50% in patients needing for target-vessel revascularization because of symptoms or signs of ischemia.

**Experimental Procedures**

Blood samples were collected from the basilic vein. Hcy levels were measured by an immunoassay method (FPIA assay, IMX system, Abbott) on plasma samples obtained after centrifuging blood collected into tubes containing EDTA. Lp (a) levels were measured on serum by an ELISA method using a commercial kit (Mercodia Apo(a) ELISA) which contains monoclonal antibodies that minimize the possible interference of heterogeneity in apo(a) isoforms on the results. PAI-1 activity levels were determined by a chromogenic method (Spectrolyse Biopool, Umea, Sweden) on plasma obtained after centrifuging blood collected into tubes containing sodium citrate. For detection of Factor V Leiden, G20210A polymorphism in the factor II gene and PAI-1 4G/5G polymorphism, genomic DNA was extracted from peripheral blood leukocytes using a MagNA Pure (Roche, Mannheim, Germany) and QUIAmp Blood Kit (QUIAGEN, Hilden, Germany) respectively. Factor V Leiden and G20210A polymorphism in the factor II gene were identified by Light-cycler capillaries method (Roche, Mannheim, Germany). PAI-1 4G/5G polymorphism was identified by PCR amplification and digestion with BslI restriction enzyme.

**Statistical analysis**

Statistical analysis was performed with SPSS (Statistical Package for Social Sciences, Chicago, USA) software for Windows (Version 11.5). Values are presented either as mean ± standard deviation (SD) or as median and range. The non-parametric Mann-Whitney test for unpaired data and chi-square test for categorical variables were used for comparisons between single groups. Fasting hyperhomocysteinemia was defined based on the 95th percentile cut-off of the control population (M=19 µmol/L; F=15 µmol/L). Elevated PAI-1 levels were defined based on the 90th percentile of the control population (14.9 IU/mol). A level of Lp(a) of 300 mg/L, which is widely accepted as the cut-off of increased vascular risk, was considered to separate normal from pathological samples. The continuous variables – Hcy, PAI-1 and Lp(a) – were divided into quintiles based on the distribution of these parameters in patients and controls (Hcy= q1:<9; q2:9.1-10.2; q3:10.3-12; q4:12.1-15.6; q5:$>$15.6 micromol/L / PAI-1= q1:<7.2; q2:7.3-9; q3:9.1-13.4; q4:13.5-20.3; q5:$>$20.3 IU/mL / Lp(a)= q1:<68; q2:69-109; q3:110-226; q4:227-410; q5:$>$410 mg/L).

To perform the multivariate analysis, unconditional logistic regression analyses were used with age (continuous variable), gender, traditional cardiovascular risk factors (hypertension, smoking habitus, diabetes, dyslipidemia and family history of CAD),
creatinine levels, thrombophilic risk factors, as the independent variables, and presence vs. absence of the disease as the dependent variables.

For the analysis of MACE in relation to the time of the event, Cox regression analysis was used with age (continuous variable), gender, traditional cardiovascular risk factors (hypertension, smoking habitus, diabetes, dyslipidemia and family history of CAD), creatinine levels, thrombophilic risk factors, ejection fraction, the number of coronary stenosed vessels and history of PCI or CABG as the independent variables.

In addition, we analyzed the interval from the hospital admission to the occurrence of MACE (uncensored observations) or to telephone interview (censored observation) in order to estimate the probability of MACE as a function of time, according to the method of Kaplan-Meier. The probability of MACE was compared among the groups with use of the log-rank test. All odds ratios are given with their 95% confidence intervals. All probability values are two-tailed with values less than 0.05 considered to be statistically significant.

Results
Clinical characteristics of patients and controls are described in table 1.
Two hundred and eighty-two (54.2%) patients were admitted to our Coronary Care Unit with diagnosis of MI, and 238 (45.8%) with unstable angina (UA). At coronary angiography, one-vessel disease was present in 364 (70%) patients, whereas two-vessel and multi-vessel disease was demonstrated in 115 (22.1%) and 41 (7.8%) patients, respectively.

Prevalence of thrombophilic risk factors
No significant differences between patients and controls were documented either in the prevalence of heterozygosity for FV Leiden or prothrombin polymorphism or in the prevalence of homozygosity for PAI-1 4G/5G polymorphism (table 2).
Hcy, Lp(a) and PAI-1 plasma levels were significantly higher in patients than in controls (table 2). Hyperhomocysteinemia was diagnosed in 115/520 patients (22.1%) and in 26/520 controls (5%). Lp(a) levels above 300 mg/L were found in 185/520 patients (35.6%) with respect to 108/520 (20.7%) controls. Finally, elevated PAI-1 levels were present in 292/520 (56.2%) patients and in 52/520 (10%) controls. No significant difference was observed in the circulating PAI-1 levels according to PAI-1 4G/5G genotype either in patients or in controls (table 3).
At multivariate regression analysis - adjusted for age, gender, creatinine levels and the traditional cardiovascular risk factors - Hcy, PAI-1 and Lp(a) within the fifth quintile with respect to the first were significant and independent risk factors for ACS: PAI-1: OR (95%CI) 24.2 (11.1-32.5), p<.0001; Hcy: = 12.1 (5.3-19.2), p<.0001; Lp(a): 3.5 (1.7-5.6), p<.005.
No significant differences in the prevalence of thrombophilic risk factors were documented between patients with unstable angina and myocardial infarction (data not shown).

Follow-up
All patients were followed up for a mean duration of 22.2 ± 3.9 months (median 24, range 12-26 (months).
At the end of follow-up, MACE were recorded in 109 patients. Fifty-four (10.3%) patients died. Fifty-two of 54 deaths were classified as due to cardiovascular causes. These included 21 acute MI, 10 sudden deaths, 10 deaths due to congestive heart failure, 10 cerebrovascular events, 1 ruptured abdominal aneurysm. Two deaths due to noncardiovascular causes were due to cancer. Forty-seven of 52 cardiovascular deaths occurred in patients with a diagnosis at admission of MI, 5/52 in patients with UA.
Clinical characteristics of patients with and without MACE are shown in table 4.
The prevalence of MI among patients with MACE was significantly higher with respect to unstable angina. On the other hand, systolic left ventricular function measured before the hospital discharge and estimated by the ejection fraction, was significantly different
between the two groups. Concerning previous revascularization procedures, a higher percentage of patients in MACE group had had a previous PCI or CABG (table 4). A similar percentage of patients with traditional cardiovascular risk factors had MACE with respect to those without (MACE in smoking vs no-smoking: 21.5% vs 25.5%; MACE in hypertension vs no-hypertension: 21.5% vs 20%; MACE in dyslipidemia vs no-dyslipidemia: 22.1% vs 22.2%; MACE in diabetes vs no-diabetes: 23.1% vs 20%; MACE in overweight vs no-overweight: 19% vs 22%). A significantly higher percentage of patients with EF ≤40% had MACE with respect to EF>40% (25.4% vs 19.8%, p<0.01).

**Thrombophilic risk factors and MACE**

Heterozygosity for FV Leiden and prothrombin polymorphism and homozygosity for PAI-1 4G/5G polymorphism, did not significantly differ in the two groups of patients (table 5). Hcy and PAI-1 plasma levels, measured at hospital admission, were significantly higher in patients with MACE (table 5) and a significantly higher percentage of patients with elevated Hcy and PAI-1 levels had MACE with respect to the others (28% vs 19%, p<.001 and 25% vs 15.7%, p<.001, respectively).

Lp(a) plasma levels were not significantly different in patients with MACE with respect to the others (table 5) and a similar percentage of patients with and without elevated Lp(a) levels had MACE (22.7% vs 20%).

At the Kaplan Meier survival curves, the overall risk of MACE was significantly higher among patients with elevated PAI-1 or Hcy levels (p=0.006 and =0.04) (fig.1 and 2) whereas no significant differences were found for Lp(a) levels or for the presence of polymorphisms investigated.

At Cox regression analysis, adjusted for age, gender, traditional cardiovascular risk factors, creatinine levels, systolic left ventricular function, the number of coronary stenosed vessels and history of PCI or CABG, Hcy and PAI-1 within the fifth quintile with respect to the first were significant and independent risk factors for the future occurrence of MACE: HCY= q1:reference group; q2:OR 3.5 (95%CI 0.3-41), p=ns; q3:OR 3.4 (95%CI 0.3-38.2),p=ns; q4:OR 3.8 (95%CI 0.5-45),p=ns; q5:OR 7.5 (95%CI 1.1-57.7), p<0.05 / PAI-1= q1:reference group; q2:OR 1.7 (95%CI 0.2-13.1), p=ns; q3:OR 2.8 (95%CI 0.5-14.1),p=ns; q4:OR 4.4 (95%CI 1.0-19.1),p=ns; q5:OR 5.3 (95%CI 1.2-23.8), p<0.05.

**DISCUSSION**

We have thoroughly investigated, for the first time, the possible role of a number of genetic and metabolic thrombophilic risk factors in the occurrence of MACE after successful coronary stenting. The main finding of our study is the demonstration that elevated PAI-1 and Hcy levels at hospital admission are independent risk factors for the future occurrence of MACE in patients with ACS who undergo PCI with stent implantation. Furthermore, we failed to find any significant role of the thrombophilic polymorphisms investigated (FV Leiden, prothrombin polymorphism, PAI 4G/5G) and of elevated Lp(a) levels as prognostic risk factors for MACE. In addition, PAI-1 4G/5G polymorphism did not significantly affect PAI-1 levels measured at the time of acute event suggesting that the influence of inflammatory state on the PAI-1 levels is scarcely genetically modulated.

A number of studies have suggested, in addition to the traditional risk factors, the existence of metabolic, hemostatic and genetic risk markers for CAD. Relationships have been found between elevated baseline values of Lp(a) [21], homocysteine [22] and PAI-1 [23] and the risk of CAD. Other studies have associated baseline PAI-1 levels with subsequent cardiac events in patients with angina, MI, or angiographic CAD.[5][6][7][8] However, elevated PAI-1 levels were shown to be predictors of new acute coronary events, including death and restenosis after coronary stenting in only two studies performed on a very low number of patients.[7][8]

Contrasting data are available on the association between Hcy levels and MACE after coronary stenting [9][10][11][12][13][14][15], whereas only two studies – with contrasting
results – have investigated the role of Lp(a) after stent implantation.[16][17] Finally, no data are present on the prevalence of thrombophilic polymorphisms – FV Leiden and prothrombin polymorphism – in patients with MACE after coronary stenting.

Data from the literature have documented that PAI-1 [24], Hcy [25] and Lp(a) [26][27][28] act as acute phase reactants. Therefore baseline levels measured at hospital admission have been influenced, at least in part, by the acute coronary event. Furthermore, our result could vary if we had assayed levels at different days after admission. Nevertheless, these levels seem to predict the future occurrence of MACE, suggesting that a more pronounced increase during the acute phase identifies a subgroup of patients at higher risk for future occurrence of new events. By determining the thrombophilic risk profile – and in particular PAI-1 and Hcy levels - of ACS patients at the time of the acute event, it is possible to select the patients who will need a more intensive follow-up after the hospital discharge. In addition, these markers may represent new potential pharmacological targets. Previous intervention trials with ACE inhibitors [29] and statins [30] have suggested a benefit of this class of drugs on the fibrinolytic system. Furthermore, it has recently been reported that thiazolidinediones, the new class of insulin sensitising drugs, are able to reduce PAI-1 protein expression [31] in human preadipocytes under basal conditions and after stimulation of the cells with TGF-beta. Accordingly, patients with insulin resistance might take benefit from this class of drugs, even in terms of reduction of PAI-1 levels.

Concerning Hcy, we and others have previously demonstrated its role as an independent risk factor for restenosis after PCI with and without stenting [11][12] [14], although other Authors [9][10] [13] failed to confirm these data. In this study, we found a significant association between hyperhomocysteinemia and MACE. Elevated Hcy levels may be easily reduced by a vitamin supplementation based on folic acid, vitamin B6 and vitamin B12 [32]. However, contrasting data are available on the possible clinical benefits resulting from the pharmacological correction of Hcy. Indeed, some Authors reported a positive effect in terms of reduced restenosis rate [33] or reduced progression of carotid intima media thickness [34] or reduced number of positive ergometric tests [35]. On the other hand, other Authors recently reported a negative effect of vitamin supplementation on the occurrence of restenosis.[10] Contradictory data might be attributed to the different dosage of vitamins used in the different studies and to the different Hcy levels in the patients enrolled or to the fact that Hcy might be only a marker of a metabolic derangement in which the leading actor is methionine instead of Hcy.[36] Intervention randomized trials designed to test the effect of Hcy-lowering therapy on multiple clinical end-points are ongoing.

An original finding of our study is the lack of a role of thrombophilic polymorphisms in the occurrence of MACE after ACS. No association was reported between FV Leiden and restenosis after PCI (with a follow-up of 6 months) without stent implantation. No data at all are available on the prevalence of prothrombin polymorphism and MACE after PCI with or without stenting. As regards MACE, it was demonstrated that FV Leiden and prothrombin polymorphism were not associated with increased susceptibility to sudden cardiac death among apparently healthy adults.[37] Our present study did not reveal independent and consistent associations between the investigated candidate genes and the risk of MACE after PCI with stent implantation, but it has to be considered that the number of MACE is probably not enough to obtain a definitive result, given the prevalences of genes investigated in the general population.

In conclusion, our study demonstrates that elevated PAI-1 and Hcy levels, but not Lp(a) and thrombophilic polymorphisms, are independent risk factors for MACE after successful coronary stenting, so that their determination at the time of the acute event could allow to select patients at higher risk for future new events.
REFERENCES
Table 1. Baseline characteristics of patients and controls

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<tr>
<th></th>
<th>Patients (n=520)</th>
<th>Controls (n=520)</th>
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<tr>
<td><strong>Age, yrs</strong>*</td>
<td>67 (32-95)</td>
<td>67 (32-89)</td>
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<tr>
<td>Males, n (%)</td>
<td>375 (72.1)</td>
<td>375 (72.1)</td>
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<td>Smoking habit, n (%)</td>
<td>279 (53.7)</td>
<td>117 (22.5) °</td>
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<tr>
<td>Hypertension, n (%)</td>
<td>311 (59.8)</td>
<td>167 (32.1) °</td>
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<tr>
<td>Diabetes, n (%)</td>
<td>134 (25.8)</td>
<td>20 (3.8) °</td>
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<tr>
<td>Dyslipidemia, n (%)</td>
<td>235 (45.2)</td>
<td>119 (22.8) °</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>177 (34)</td>
<td>49 (9.4) °</td>
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<td>Creatinine (mg/dl)</td>
<td>0.95±0.3</td>
<td>0.93±0.2</td>
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<td>BMI (kg/m²)^</td>
<td>25.8±2.8</td>
<td>24.5±3.1°</td>
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<tr>
<td>History of MI</td>
<td>18 (3.5)</td>
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<td>PCI</td>
<td>38 (7.3)</td>
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<td>CABG</td>
<td>17 (3.3)</td>
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* Median and range; ^Mean ± Standard Deviation; BMI=Body Mass Index; ° p < 0.0001
Table 2. Thrombophilic risk factors

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<th>Patients (n=520)</th>
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<tr>
<td>Hcy levels (µmol/L)*</td>
<td>12.7 (3-95)</td>
<td>10 (6-24)*</td>
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<tr>
<td>PAI-1 levels (UI/mL)*</td>
<td>16 (1-64)</td>
<td>8 (4-38)*</td>
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<tr>
<td>Lp(a) levels (mg/L)*</td>
<td>170 (1-2141)</td>
<td>135 (7-1390)</td>
<td>.02</td>
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<tr>
<td>Heterozygosity for Factor V Leiden, n (%)</td>
<td>20 (3.8)</td>
<td>20 (3.8)</td>
<td>.99</td>
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<tr>
<td>Heterozygosity for Factor II G20210A, n (%)</td>
<td>21 (4.0)</td>
<td>19 (3.6)</td>
<td>.87</td>
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<tr>
<td>Homozigosity for PAI-1 4G/5G, n (%)</td>
<td>166 (31.9)</td>
<td>156 (30)</td>
<td>.55</td>
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*Median and range
Table 3. PAI-1 Activity (IU/ml) according to PAI-4G/5G genotype

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<tr>
<th>PAI-1 4G/5G POLYMORPHISM</th>
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<td>5G5G</td>
<td>15.2</td>
<td>16.0</td>
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<tr>
<td>Patients</td>
<td>(1-64)</td>
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<td></td>
<td>0.33</td>
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<td>Controls</td>
<td>7.9</td>
<td>8.0</td>
<td>8.3</td>
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<tr>
<td>(n=520)</td>
<td>(4-35)</td>
<td>(4-38)</td>
<td>(4-36)</td>
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*Median and range
Table 4. Clinical characteristics of patients according to the occurrence of MACE

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<td>No (n=411)</td>
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<td>67 (35-95)</td>
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<td>Males, n (%)</td>
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<td>289 (70.3)</td>
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<td>64 (58.7)</td>
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<td>BMI&gt;25 kg/m², n (%)</td>
<td>50 (45.8)</td>
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<td>Ejection Fraction (%)</td>
<td>45±12.6</td>
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Diagnosis at admission

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<td>Unstable Angina</td>
<td>41 (37.6)</td>
<td>197 (47.9)</td>
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<td>-</td>
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<td>PCI</td>
<td>31 (28.4)</td>
<td>7 (1.7)</td>
<td>0.001</td>
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<td>CABG</td>
<td>12 (11)</td>
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* Median and range
Table 5. Thrombophilic risk factors according to the occurrence of MACE

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<th>NO MACE (n=411)</th>
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<td>Hcy (umol/L)*</td>
<td>16 (3-95)</td>
<td>12.3 (3.3-90.7)</td>
<td>.001</td>
</tr>
<tr>
<td>PAI-1 (UI/mL)*</td>
<td>19 (2-48)</td>
<td>16 (1-64)</td>
<td>.001</td>
</tr>
<tr>
<td>Lp(a)*</td>
<td>191.5 (1-1962)</td>
<td>168 (2-2141)</td>
<td>0.8</td>
</tr>
<tr>
<td>Heterozygosity for Factor V Leiden, n (%)</td>
<td>4 (3.7)</td>
<td>16 (3.9)</td>
<td>0.8</td>
</tr>
<tr>
<td>Heterozygosity for Factor II G20210A, n (%)</td>
<td>4 (3.7)</td>
<td>17 (4.1)</td>
<td>0.8</td>
</tr>
<tr>
<td>Homozygosity for PAI-14G/5G polymorphism, n(%)</td>
<td>37 (33.9)</td>
<td>129 (31.4)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Median and range
PAI-1 < 14.9 IU/mL

PAI-1 > 14.9 IU/mL

P = 0.006 (log rank test)
Hyperhomocysteinemia

Homocysteine levels within the normal range

Event-Free Rate

Follow-up (months)

P = 0.04 (log rank test)