

Hemostatic and fibrinolytic parameters in survivors of myocardial infarction: a low plasma level of plasmin– α_2 -antiplasmin complex is an independent predictor of coronary re-events

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(Received 19 May 2000; revised 5 October 2000; accepted 5 October 2000)

Abnormalities of coagulation or fibrinolysis play a role in the pathogenesis of coronary artery disease (CAD). Elevated plasma levels of fibrinogen, von Willebrand factor antigen, plasminogen activator inhibitor-1 and tissue-type plasminogen activator were reported to be predictive for reinfarction and death in patients with CAD. We investigated the risk for coronary re-events associated with 18 hemostatic and fibrinolytic parameters in a prospective study including 200 survivors of myocardial infarction (MI). During a 2-year follow-up, 37 patients suffered one of the following predefined re-events: fatal MI ($n=2$), non-fatal MI ($n=5$), percutaneous transluminal coronary angioplasty ($n=17$) or coronary artery bypass grafting ($n=13$). Low plasmin– α_2 -antiplasmin complex (PAP) plasma levels were associated with an up to fivefold (95% confidence interval, 1.6–15.3) increase in relative risk. The association between decreasing PAP levels and coronary re-events remained significant ($P=0.004$) after correction for possible confounders using multiple logistic regression analysis. Our data indicate low PAP plasma levels to be associated with subsequent coronary events in patients with a history of MI. *Blood Coagul Fibrinolysis* 12:17–24 © 2001 Lippincott Williams & Wilkins.

Keywords: myocardial infarction, blood coagulation, fibrinolysis, prediction, plasmin– α_2 -antiplasmin

Introduction

Myocardial infarction and angina pectoris are very common in Western countries. Several studies have demonstrated the pathogenetic role of local thrombus formation in coronary arteries at the site of a ruptured plaque [1–3]. Plaque disruption leads to platelet activation and thrombin generation [1,4,5]. Abnormalities of the coagulation system, mainly

increased plasma concentrations of fibrinogen and factor VII, were identified to contribute to coronary thrombosis. These results are derived from prospective studies on healthy subjects [6–8]. One investigator reported elevated plasmin– α_2 -antiplasmin levels (PAP) to be predictive for myocardial infarction in healthy elderly individuals [9]. Another

Sponsorship: This study was supported by grants from the Swiss National Foundation for Scientific Research (number 3200-55312.98) and from the Gesellschaft für Thrombose und Hämostaseforschung, Germany. M. Redondo, T. Mauron, F. Demarmels Biasiutti and B. Lämmle are with the Central Hematology Laboratory, University Hospital Bern, Bern, Switzerland; W. A. Wuillemin is with the Department of Internal Medicine, Division of Hematology, Kantonsspital Lucerne, Switzerland; and V. A. Carroll and B. R. Binder are with the Department of Vascular Biology and Thrombosis Research, University of Vienna, Austria. Address correspondence to Walter A. Wuillemin, M.D., Ph.D., Department of Internal Medicine, Division of Hematology, Kantonsspital Lucerne, CH-6000 Lucerne 16, Switzerland. Tel: (+41) 41 205 51 47; fax: (+41) 41 205 51 09; e-mail: walter.wuillemin@ksl.ch

report showed an increased fibrinolytic activity measured by PAP in patients with post-infarction angina, new onset of unstable angina and crescendo angina compared with healthy controls [10]. Patients surviving a first myocardial infarction deserve consideration as a special group. They have a cardiac death rate of about 6% and a non-fatal reinfarction rate of 4–5% in the first year after discharge [11]. A large European multicentre study [12] and several cohort studies [13–18] on patients with previous myocardial infarction or angina pectoris showed elevated plasma levels of fibrinogen, von Willebrand factor antigen (vWF:Ag), plasminogen activator inhibitor type 1 (PAI-1) and tissue-type plasminogen activator (t-PA), as well as dyslipoproteinemia to be predictive for subsequent coronary events in these patients. For an appropriate clinical management it is important to identify patients with an elevated risk for subsequent coronary events.

The purpose of the present study was to investigate haemostatic and fibrinolytic parameters as possible predictors of subsequent coronary events in a 2-year follow-up in 200 survivors of myocardial infarction.

Materials and methods

Study population

Two hundred Caucasian patients (174 males, 26 females) were consecutively selected from the data files of the Division of Cardiology of the University Hospital. The inclusion/exclusion criteria were: (i) history of acute myocardial infarction having occurred at least 2 months before investigation, (ii) stable coronary artery disease, and (iii) no concomitant disease with a life expectancy of less than 6 months. Myocardial infarction was diagnosed based on typical electrocardiographic changes and raised biochemical markers such as creatine kinase and/or troponin T.

All patients except two had undergone coronary angiography in the time period after the qualifying myocardial infarction and study inclusion. One-, two-, or three-vessel disease was present in 33.8, 36.4 and 26.8, respectively, whereas 3% showed angiographically normal coronary arteries. Patients were considered as smokers if they had smoked cigarettes for more than 5 pack-years. Arterial hypertension and diabetes mellitus were diagnosed according to the patient's history and to their medical treatment. Body mass index (BMI) was available from all patients. Blood was collected in the morning in a fasting state, drawn from an

antecubital vein using a 19-gauge butterfly needle, and was collected into two 10 ml plastic syringes (Monovette[®]; Sarstedt, Nümbrecht, Germany), each containing 1 ml of 0.106 mol/l trisodium citrate, and into a 5.5 ml plastic syringe (Monovette[®]) containing 0.5 ml of 0.45 mol/l acidified sodium citrate. Plasma was prepared by twice centrifuging at 1500 x *g* for 10 min each at 15–18°C and then stored in polypropylene tubes at –70°C. After a follow-up period of 2 years, 177 participants were re-investigated at our outpatient clinic and 17 participants were interviewed by phone. Three participants were lost to follow-up, two participants were not willing to answer the questions, and one participant had died from cancer of the oesophagus. Death and the following pre-defined coronary re-events during the follow-up period were recorded: myocardial infarction, coronary artery bypass graft surgery (CABG) and percutaneous transluminal coronary angioplasty (PTCA). Events were ascertained by questionnaire and hospital reports. Furthermore, medical treatment, weight and height were recorded and, where appropriate, relatives and family doctors were asked about death and cause of death. The study had been approved by the Ethics Committee of the University of Bern.

Determination of coagulation and fibrinolytic parameters

Clotting activities of factor XI (FXI:C), factor XII (FXII) and high molecular weight kininogen (HK) were measured by an activated partial thromboplastin time-based assay using Neothromtin[®] (ellagic acid–phospholipid mixture; Behring, Marburg, Germany) as previously described [19]. FXII antigen levels were measured by a dot immunobinding assay as reported elsewhere [20]. Plasma levels of prekallikrein (PK) were assessed by measuring its amidolytic activity using a chromogenic substrate assay as previously described [21]. Clotting activities of factors II, V, VII and X (FII:C, FV:C, FVII:C and FX:C, respectively) were measured by a prothrombin time-based coagulation assay using Thromborel S[®] (Behring) and the respective human-deficient substrate plasma. FII:C, FVII:C and FX:C were measured only in patients without oral anticoagulation. Fibrinogen was measured according to the method of Clauss [22]. vWF:Ag was assayed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Dako, Glostrup, Denmark). PAI-1 active antigen and total PAI-1 antigen were measured using ELISA techniques [23]. t-PA antigen was determined using a bioimmunoassay [24]. PAP levels were determined using a

sandwich immunoassay (Technoclone, Vienna, Austria) [25]. Protein C inhibitor (PCI) active antigen was measured using a functional immunological assay [26].

FV nucleotide 1691 genotype and prothrombin gene 20210G → A transition

FV 1691 genotype predicting amino acid 506 [27] was identified by polymerase chain reaction (PCR) technique and MnlI restriction as previously described [28,29]. The prothrombin gene 20210G → A transition was assessed by PCR technique and HindIII restriction as described elsewhere [30].

Other assays

White blood cell count was measured using ethylenediamine tetraacetic acid–blood on a STKR (Culter, Miami, Florida, USA) and total cholesterol were determined using serum on a Hitachi 717 (Hitachi, Tokyo, Japan).

Statistics

Median and range or proportions for cardiovascular risk factors were calculated for patients without an event and for patients with an event during the follow-up. Comparison of continuous variables between event group and non-event group was performed by Mann–Whitney *U* test, and comparison of categorical variables by chi-square test or Fisher’s exact test, where appropriate. *P* values were two-tailed and considered significant below 0.05. Confidence intervals (CI) were calculated at the 95% level. Statistical analysis was performed using Sigma-stat (Jandell, Erkrath, Germany). Crude odds ratios were calculated by standard methodology as estimates of the relative risk for events during the 2-year follow-up for the respective coagulation and fibrinolytic factors, and for the genetic mutations. In addition, we used simple logistic regression analysis (SAS package, release 6.12; SAS Institute Inc., Cary, North Carolina, USA) to search for a possible association between a respective coagulation and fibrinolytic factor and subsequent coronary re-events, and used multiple logistic regression analysis for adjustment of effects of other coronary risk factors. Adjustment was made for the continuous risk factors age, BMI, total cholesterol, fibrinogen and for the dichotomized risk factors sex, smoking habit (yes/no), arterial hypertension (yes/no) and diabetes mellitus (yes/no).

Results

Baseline characteristics in the event and non-event groups

Thirty-seven patients suffered one of the following pre-defined coronary events during the 2-year follow-up period: fatal myocardial infarction (*n* = 2), non-fatal myocardial infarction (*n* = 5), PTCA (*n* = 17), and CABG (*n* = 13) (Table 1). Two patients had twice undergone PTCA, and one patient had a myocardial infarction and a PTCA 7 months later. These 37 patients constitute the event group. The median time between study inclusion and occurrence of the re-event was 9 months (range, 1–21 months). Table 2 shows the baseline characteristics of the patients with and without an event, respectively.

Fibrinogen, FII:C, FV:C, FVII:C, FX:C and vWF:Ag

As presented in Table 3, we found slightly higher plasma levels of fibrinogen and of vWF:Ag in the event group as compared with the non-event group. The differences were, however, not significant. Furthermore, no significant differences between the two groups were found regarding the plasma levels of FII:C, FV:C, FVII:C and FX:C.

Table 1. Follow-up overview and events during the 2-year follow-up

	Number of patients
Included in the study	200
Lost for follow-up	6
Not found	3
Not willing to answer	2
Non-vascular death*	1
Non-event group	157
Event group	37
Fatal myocardial infarction	2
Non-fatal myocardial infarction	5
Percutaneous transluminal coronary angioplasty	17
Coronary artery bypass grafting	13

Each subject of the event group was classified once even if having suffered more than one clinical endpoint during follow-up. One patient classified only in the non-fatal myocardial infarction subgroup had one myocardial infarction and a percutaneous transluminal coronary angioplasty (PTCA). Two patients classified in the percutaneous transluminal coronary angioplasty subgroup had each twice undergone PTCA. *Oesophageal carcinoma.

Table 2. Baseline characteristics among patients with an event (event group) and among patients without an event (non-event group) during follow-up

	Event group (n = 37)	Non-event group (n = 157)	P value*
Age (years)	59 (39–68)	57 (32–72)	0.26
Male sex (%)	86.5	87.9	0.97
Smoker (%)	67.6	74.5	0.51
Hypertension (%)	48.6	46.5	0.96
Diabetes mellitus (%)	29.7	17.8	0.16
Body mass index (kg/m ²)	26.4 (22.4–39.0)	26.0 (20.6–43.3)	0.28
Total cholesterol (mmol/l)	5.78 (4.02–9.75)	6.00 (3.09–9.56)	0.56
Leucocytes (x 10 ⁹ /l)	7.4 (4.3–15.9)	7.1 (3.9–11.9)	0.42
MI > 1 (%)	16.2	15.9	0.99
Two- and three-vessel disease (%)	69.5	63.3	0.62
Oral anticoagulation (%)	37.8	31.2	0.20

Continuous data are presented as median (range). Myocardial infarction (MI) > 1, More than one myocardial infarction before inclusion in the study. *Mann–Whitney *U* test or chi-square test.

Table 3. Coagulation factors, contact activation factors, fibrinolytic and fibrinolysis-inhibitory proteins in the event and non-event groups

	Event group (n = 37)	Non-event group (n = 157)	P value*
Fibrinogen (g/l)	3.0 (1.9–4.3)	2.8 (1.9–4.8)	0.19
FII:C (%)	94 (73–118)	96 (41–127)	0.73
FV:C (%)	110 (87–138)	110 (69–186)	0.98
FVII:C (%)	116 (81–172)	118 (66–204)	0.79
FX:C (%)	108 (84–147)	108 (28–138)	0.66
vWF:Ag (%)	116 (58–224)	113 (47–330)	0.70
FXII:C (%)	99 (44–133)	96 (41–167)	0.40
FXII:Ag (%)	104 (55–155)	96 (45–201)	0.14
FXI:C (%)	110 (72–185)	111 (56–190)	0.67
PK:Am (%)	120 (82–172)	112 (76–168)	0.21
HK:C (%)	110 (74–159)	107 (74–162)	0.69
t-PA (ng/ml)	9.6 (3.5–39.3)	9.9 (2.1–94.4)	1.00
Active PAI-1 (U/ml)	10.4 (1.6–34.9)	8.7 (0.5–46.4)	0.34
Total PAI-1 (ng/ml)	29.6 (4.5–99.2)	27.5 (2–113.6)	0.34
Active PCI (%)	149 (0–365)	125 (0–1000)	0.19
PAP (ng/ml)	84 (0–278)	111 (0–1682)	0.01

Continuous data are presented as median (range). Clotting activities of factors II, VII and X (FII:C, FVII:C and FX:C, respectively) were measured only in non-anticoagulated patients (n = 131). FV:C, FXII:C, FXI:C, Clotting activities of factors V, XII and XI, respectively; vWF:Ag, von Willebrand factor antigen; FXII:Ag, factor XII antigen; PK:Am, prekallikrein amidolytic activity; HK:C, clotting activity of high molecular weight kininogen; t-PA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor-1; PCI, protein C inhibitor; PAP, plasmin- α_2 -antiplasmin. *Mann–Whitney *U* test.

Contact activation factors

The plasma levels of the contact activation proteins FXII, FXI, PK and HK are presented in Table 3. With the exception of FXI:C, higher plasma levels of the contact activation factors were found in the event group as compared with the non-event group. However, the differences were not significant.

Fibrinolytic and fibrinolysis-inhibitory parameters

Plasma levels of t-PA, active PAI-1, total PAI-1, active PCI and PAP for patients with and without an event, respectively, are presented in Table 3. t-PA antigen plasma levels were not different in the two groups ($P = 1.00$). Plasma levels of active PAI-1 ($P = 0.34$), total PAI-1 ($P = 0.34$) and active PCI

($P = 0.19$) were slightly higher in the event group as compared with the non-event group. However, the differences were not significant. Significantly lower PAP levels ($P = 0.01$) were observed among the patients with an event as compared with those without an event (Table 3). No difference was found for PAP levels between anticoagulated and non-anticoagulated patients ($P = 0.89$).

FV R506Q mutation and prothrombin gene 20210G → A transition

The FV Leiden mutation was detected only in the heterozygous form and showed a similar prevalence ($P = 0.76$) in the event (5.4%) and non-event group (5.7%). One individual of the non-event group and none of the event group showed a heterozygous prothrombin gene 20210G → A transition. Neither of these two genetic markers was associated with an increased relative risk for subsequent coronary events during the follow-up period.

Relative risk analysis

Next, we investigated whether one of the haemostatic or fibrinolytic parameters would be associated with an increased relative risk for a subsequent coronary event. We first calculated crude odds ratios

for the three upper quartiles as compared with the lowest quartile for each parameter. The crude odds ratios for PAP were calculated for the first (< 68 ng/ml), second (69–111 ng/ml) and third quartile (112–184 ng/ml) as compared with the fourth quartile (> 185 ng/ml). Second, we sought any possible significant association between the respective parameter and a coronary event using a simple logistic regression model. Table 4 shows that only for low PAP levels was a significant association with re-events found, whereas no significant result was seen for the other parameters. We analyzed the relative risk of low PAP levels using various cut-off values for PAP complexes (Table 5). Plasma levels of PAP ≤ 100 ng/ml were associated with a 2.2-fold (95% CI, 1.1–4.6) increase in risk for subsequent coronary events. The relative risk increased with lower cut-off levels showing a 5.0-fold (95% CI, 1.6–15.3) increase in risk for coronary events for patients with undetectable PAP levels as compared with those with PAP levels above 0 ng/ml. Analysis using a simple logistic regression model including PAP as a continuous variable confirmed the significant ($P = 0.02$) association between decreasing PAP levels and the risk for a subsequent coronary event. Moreover, this association remained significant

Table 4. Relative risk for a coronary event of various coagulation factors, contact activation factors and fibrinolytic and fibrinolysis-inhibitory proteins

	Quartile				<i>P</i> value*
	First	Second	Third	Fourth	
Fibrinogen (g/l)	1.0	0.6 (0.19–2.08)	1.6 (0.61–4.29)	1.3 (0.47–3.70)	0.45
FII:C (%)	1.0	1.0 (0.30–3.52)	1.0 (0.27–3.38)	0.7 (0.17–2.65)	0.96
FV:C (%)	1.0	1.6 (0.57–4.46)	1.6 (0.55–4.49)	1.0 (0.30–2.96)	0.84
FVII:C (%)	1.0	0.9 (0.25–2.89)	0.6 (0.16–2.27)	0.9 (0.25–2.89)	0.71
FX:C (%)	1.0	4.2 (0.82–21.18)	3.8 (0.74–19.91)	1.9 (0.32–11.45)	0.44
vWF: Ag (%)	1.0	1.2 (0.28–4.79)	1.9 (0.50–6.90)	1.4 (0.36–5.50)	0.83
FXII:C (%)	1.0	2.1 (0.64–6.57)	2.7 (0.90–8.30)	1.6 (0.47–5.19)	0.59
FXII:Ag (%)	1.0	1.3 (0.39–4.55)	3.0 (0.99–9.06)	2.1 (0.64–6.57)	0.30
FXI:C (%)	1.0	1.9 (0.66–5.24)	0.8 (0.25–2.64)	1.6 (0.58–4.57)	0.47
PK:Am (%)	1.0	0.3 (0.08–1.34)	2.2 (0.83–5.67)	1.3 (0.46–3.60)	0.25
HK:C (%)	1.0	1.0 (0.35–2.90)	1.2 (0.41–3.24)	1.2 (0.42–3.33)	0.70
t-PA (ng/ml)	1.0	1.2 (0.43–3.19)	1.1 (0.43–3.19)	0.9 (0.31–2.54)	0.85
Active PAI-1 (U/ml)	1.0	0.7 (0.22–2.10)	0.9 (0.31–2.60)	1.6 (0.62–4.11)	0.29
Total PAI-1 (ng/ml)	1.0	0.7 (0.24–2.02)	0.9 (0.35–2.59)	1.1 (0.43–2.96)	0.27
Active PCI (%)	1.0	0.9 (0.29–2.64)	1.3 (0.46–3.59)	1.6 (0.58–4.29)	0.76
PAP (ng/ml)	2.3 (0.86–6.18)	0.8 (0.25–2.64)	1.0 (0.31–3.04)	1.0	0.02

Data are presented as odds ratios (95% confidence interval) for values in the second, third and fourth quartiles as compared with the first quartile. PAP data are presented as odds ratios (95% confidence interval) for values in the first, second and third quartile as compared with the fourth quartile. Clotting activities of factors II, VII and X (FII:C, FVII:C, FX:C, respectively) were measured only in non-anticoagulated patients ($n = 131$). FV:C, FXII:C, FXI:C, Clotting activities of factors V, XII and XI, respectively; vWF:Ag, von Willebrand factor antigen; FXII:Ag, factor XII antigen; PK:Am, prekallikrein amidolytic activity; HK:C, clotting activity of high molecular weight kininogen; t-PA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor-1; PCI, protein C inhibitor; PAP, plasmin- α_2 -antiplasmin. *The P value is taken from the simple logistic regression model (each parameter was introduced in the model as a continuous variable).

Table 5. Plasmin- α_2 -antiplasmin complexes: different cut-off values and relative risk for coronary events during follow-up

Cut-off (ng/ml)	Event group (n)	Non-event group (n)	Odds ratio (95% confidence interval)	
			Without adjustment	With adjustment*
> 100	14	90	1.0	
≤ 100	23	67	2.2 (1.1–4.6)	3.5 (1.5–8.1)
> 50	20	128	1.0	
≤ 50	17	29	2.7 (1.2–5.8)	3.4 (1.5–8.0)
> 10	29	148	1.0	
≤ 10	8	9	4.5 (1.6–12.7)	5.1 (1.7–15.6)
> 0	30	150	1.0	
0	7	7	5.0 (1.6–15.3)	5.5 (1.6–18.5)

*Adjustment was made for age, sex, smoking habit, arterial hypertension, diabetes mellitus, body mass index, total cholesterol, and fibrinogen.

($P = 0.004$) after adjustment for possible confounders such as age, sex, BMI, smoking habit, arterial hypertension, diabetes mellitus, total cholesterol and fibrinogen in a multiple logistic regression model.

Discussion

The objective of the present study was to investigate a possible association between baseline measurements of haemostatic and fibrinolytic factors indicating the existence of a prothrombotic state or an alteration of the coagulation and/or fibrinolytic system, and the occurrence of coronary re-events during a 2-year follow-up in patients with a history of myocardial infarction.

Our main finding was a strong and independent association of low plasma levels of PAP with the occurrence of a coronary event during the 2-year follow-up. Low plasma levels of PAP were associated with an up to 5.0-fold (95% CI, 1.6–15.3) increase in risk for a coronary event. This association remained significant after adjusting for possible confounders such as age, sex, BMI, smoking habit, arterial hypertension, diabetes mellitus, total cholesterol and fibrinogen. To the best of our knowledge, this is the first report showing low plasma levels of PAP to be independently predictive for subsequent coronary events in survivors of myocardial infarction. This finding is in line with an earlier observation that, in the non-anticoagulated group of patients (including the event and non-event groups), baseline plasma levels of PAP were significantly lower than in a healthy control group [26]. However, a recent study reported elevated PAP levels to be predictive for myocardial infarction in elderly healthy subjects [9] and another study showed elevated PAP levels in post-infarction angina, new

onset of angina or crescendo angina as compared with healthy individuals, respectively [10]. Another report showed elevated PAP levels to be a risk factor for cardiovascular disease in elderly patients and hypothesized elevated PAP levels to be closely associated with atherosclerosis and inflammation [31]. This observation that low as well as elevated PAP levels were found to be associated with coronary events is difficult to interpret. PAP levels may reflect both plasmin generation and fibrin formation, and therefore elevated PAP levels may indicate not only fibrinolysis, but also ongoing clot formation and degradation, probably depending on the study population and its disease state. Therefore, elevated PAP levels could, in patients with unstable angina [10], indicate ongoing clot formation with subsequent coronary events, whereas in another study [26], as well as among our patients with stable coronary artery disease at the time of investigation, low PAP levels may indicate low constitutional fibrinolytic activity. This hypothesis may further be substantiated by our finding of slightly higher plasma levels of PAI-1 in the event group. This is in agreement with other reports, showing elevated plasma levels of PAI-1 [14–16] to be predictive for coronary events in patients suffering from coronary artery disease. Altogether, our results may indicate the presence of a reduced fibrinolytic capacity in patients who will suffer a future coronary event. However, given the complex linking role for fibrinolysis between inflammation and atherosclerosis [31], this hypothesis has to be considered as preliminary.

An alternative explanation for our finding could be chance alone, since more than 20 haemostatic, fibrinolytic, and metabolic parameters were evaluated. In order to address this point, we performed

multiple logistic regression analysis including all parameters measured. In this model (data not shown), age ($P = 0.03$), total cholesterol ($P = 0.03$) and PAP levels ($P = 0.02$) were found to be significantly associated with coronary re-events. This analysis strengthens our main finding of low PAP levels to be associated with coronary re-events.

We found slightly higher plasma levels of the contact activation proteins (FXII, PK, HK), fibrinogen, vWF, active PAI-1, total PAI-1, and active PCI in patients with a subsequent coronary event as compared with those without. However, the differences did not reach significance. The white blood cell count was slightly higher in the event group as compared with the non-event group; however, this was not predictive for coronary events. This is in contrast to other reports showing higher levels of white blood cells in those patients suffering from coronary artery disease who will die within 1 year as compared with those who will survive [13]. For t-PA and the other haemostatic parameters, no difference was observed between the event and the non-event groups.

In conclusion, our findings indicate baseline plasma levels of PAP to be lower in survivors of myocardial infarction who will suffer from a subsequent coronary event as compared with those with uneventful follow-up. A low PAP plasma level is an independent predictor for subsequent coronary events during a 2-year follow-up. Further prospective studies among patients suffering from coronary artery disease are needed to establish the predictive value of PAP plasma levels among various patient populations.

Acknowledgments

The authors thank Irmela Sulzer and Daniela Spina for technical support.

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