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Plasminogen Activator Inhibitor and the Risk of Cardiovascular Disease: The Framingham Heart Study

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Abstract

Introduction—Although plasminogen activator inhibitor (PAI-1) plays a key regulatory role in fibrinolysis, it has not been clearly shown to independently predict cardiovascular disease (CVD) among individuals without prior CVD. We investigated, in the Framingham Heart Study offspring cohort, whether PAI-1 predicted CVD risk among individuals without prior CVD.

Methods—Plasma PAI-1 antigen and Tissue plasminogen activator (TPA) antigen were measured in 3203 subjects without prior CVD between 1991-95; average follow-up of 10 years. PAI-1 was remeasured 4 years after baseline, to determine the effect of serial change on risk.

Results—PAI-1 levels (mean ± SD) were 29.1 ng/ml (19.2) versus 22.1 (16.5) for those and without incident CVD; p<0.001, and TPA levels were 12.0 ng/ml (5.7) versus 9.0 (4.7); p<0.001. PAI-1 and TPA antigen levels had a strong unadjusted linear relation with incident CVD (p<0.001). After adjustment for conventional risk factors, the hazards ratios (HR) for higher quartiles of PAI-1, compared with the lowest, were 1.9, 1.9, 2.6 (linear trend p=0.006), and 1.6, 1.6, 2.9 (p<0.001) for TPA antigen. The adjusted HRs for increasing quartiles of serial change in PAI-1 at 4 years, compared with the lowest, were 0.9, 0.8, 1.3 (p=0.050). C statistic assessment showed that adding PAI-1 or TPA to conventional risk factors resulted in small increases in

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

discrimination and modest reclassification of risk, which was statistically significant for TPA (net reclassification 6.8%, p=0.037) but not PAI-1 (4.8%, p=0.113).

Conclusion—PAI-1 and TPA antigen levels are predictive of CVD events after accounting for established risk factors. A serial increase in PAI-1 is associated with a further increase in risk. These findings support the importance of fibrinolytic potential in CVD.

Keywords

Plasminogen activator inhibitor 1; Tissue plasminogen activator; Myocardial Infarction; Cardiovascular Diseases

INTRODUCTION

The endogenous fibrinolytic system plays an important role in the regulation of fibrin deposition in vessel walls, vascular remodeling, and thrombosis. The link between the major inhibitor of the fibrinolytic system, plasminogen activator inhibitor (PAI-1) and cardiovascular disease (CVD) risk, has therefore, been the focus of significant research.[1] Although PAI-1 has been shown to be a predictor of recurrent CVD,[2] PAI-1 has not been clearly shown to be an independent predictor of CVD in healthy individuals.[3,4] In part, this is due to its close relationship with other risk factors that attenuate statistical significance for PAI-1 after adjustment.[5]

Tissue plasminogen activator (TPA) antigen has been more convincingly demonstrated than PAI-1 to be an independent risk factor for CVD [6,7], although Lowe and colleagues, who performed a meta-analysis, concluded that additional studies were needed to determine the extent of independence of TPA from other risk factors.[8] Furthermore, the role of TPA antigen remains unclear because it in large part reflects circulating TPA-PAI-1 complexes, and increased concentration of TPA antigen indicates reduced fibrinolytic capacity.[9]

We hypothesized that impaired fibrinolytic potential, as evidenced by elevated levels of PAI-1 antigen and TPA antigen would be predictive of CVD in a community–based cohort of individuals free from known CVD. In addition, we hypothesized that a temporal increase in level of PAI-1 during follow-up would be associated with a higher CVD risk, after adjusting for baseline biomarker levels.

MATERIALS AND METHODS

Study Subjects

The Framingham Heart Study (FHS) is a community-based, prospective observational study of CVD and its risk factors. The FHS offspring cohort began recruitment in 1971 by enrolling children of the original FHS cohort and the children's spouses. Members of the offspring cohort are white and of mixed European ancestry.

Participants attending the fifth examination cycle (1991 through 1995) of the FHS offspring cohort were eligible for inclusion in this study. Data collected at examination 5 and, if available, at examination 6 (1995 through 1998) were used in the analysis. The institutional

review board of Boston University Medical Center approved the protocol, and participants provided written informed consent. During the fifth examination cycle (the baseline examination for the present study) 3799 participants underwent a standardized medical history, physical examination, 12-lead ECG, and laboratory assessment of CVD risk factors. We assessed the participants for cigarette smoking and diabetes mellitus and measured blood pressure, body-mass index, total cholesterol levels and high-density lipoprotein (HDL) cholesterol levels. Medication use, including antihypertensive therapy, was recorded. We measured PAI-1 and TPA antigen levels in 3564 participants. For the incident analysis of participants attending the fifth examination cycle, we excluded 342 participants with prevalent CVD (myocardial infarction, angina pectoris, congestive heart failure or stroke) and a further 19 participants with missing covariate information, leaving 3203 patients for analysis. For the evaluation of the effect of serial changes of PAI-1 on incident disease, we excluded an additional 62 participants who did not attend cycle 6 as well as 27 participants who attended cycle 6 but did not have PAI-1 measured, 361 participants with prevalent CVD at Cycle 6, and 18 participants with missing covariate data at cycle 6, leaving 2735 participants for the serial change analysis. PAI-1 was our primary measure of interest because of its mechanistic effect, so TPA was not also measured for serial change.

Clinical Definitions and Laboratory Methods

We measured height and weight with the subject standing in light clothes. Body mass index was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). Blood pressure values were taken as the mean of 2 measures. We defined diabetes mellitus as a fasting blood glucose >125mg/dl, or treatment with glucose lowering medication. Those who reported smoking cigarettes regularly during the year before the examination were considered current smokers. Blood samples were collected from an antecubital vein in the morning, after an overnight fast and after participants had been supine for approximately 10 minutes. Specimens for measurement of PAI-1 antigen and TPA antigen were collected in tubes with 3.8% sodium citrate (9:1vol/vol) and kept on ice until centrifugation at 2500g for 30 minutes at 4°C. Plasma aliquots were quickly frozen and stored at −70°C for subsequent analysis. PAI-1 antigen levels were determined by a commercially available sandwich enzyme-linked immunosorbent assay according to the description of Declerck et al (TintElize PAI-1, Biopool AB).[10] Levels of TPA antigen also were obtained using an enzyme-linked immunosorbent assay (TintElize TPA, Biopool AB) following a procedure described by Ranby et al.[11] The intra-assay coefficient of variation in our laboratory was 8.1% for PAI-1 and 5.5% for TPA. Laboratory analyses were conducted when sufficient samples were obtained to fill the plates for the ELISA assays, within 3 months of sampling and freezing. The same assay technique, including calibration curves, were used by the same research personnel, together with quality control measures to ensure stability of results. For determination of lipids, blood was anticoagulated with EDTA at a final concentration of 1mg/mL. Plasma was separated by centrifugation at 2500g for 30 minutes at 4°C. Lipid measurements were made in fresh specimens. HDL cholesterol was measured after precipitation of LDL and VLDL cholesterol with dextran-magnesium. Plasma levels of total cholesterol, HDL and triglycerides were measured by automated enzymatic methods with an Abbot Diagnostics ABA-200 bichromatic analyzer and Abbot A-Gent enzymatic reagents. LDL cholesterol was calculated by the Friedwald equation in all

cases with triglycerides levels <500 mg/dL. The Framingham laboratory participates in the Centers for Disease Control and Prevention's lipoprotein cholesterol laboratory standardization program.

Outcome

The primary outcome was a major cardiovascular event, defined as a composite of fatal and nonfatal myocardial infarction, coronary insufficiency (prolonged angina with documented electrocardiographic changes), heart failure and stroke. All suspected events were reviewed by a committee of three investigators, using previously described criteria.[12]

Statistical Analysis

We used separate multivariable Cox proportional-hazards models to examine the association of examination cycle 5 PAI-1 and TPA levels with the risk of CVD events. For each biomarker, participants were categorized into quartiles according to the value of the biomarker. Hazard ratios (HR) of each higher quartile versus the lowest (first) quartile were determined, first adjusted for age and then adjusted for age, sex and conventional risk factors of systolic blood pressure, diabetes mellitus, smoking, total cholesterol, HDL cholesterol and triglycerides. Tests of the significance for linear and quadratic relationships between each log-transformed biomarkers and risk of CVD were carried out. Men and women were combined as there was no significant interaction of markers with sex. Similar analytical methods were used to examine the association of change in PAI-1 from examination cycle 5 to cycle 6 with the risk of CVD events. In addition to using the first quartile as the index group for serial analysis, we also analyzed the second quartile as the index group, because the first quartile had higher initial basal levels. The discriminatory ability of the Cox models was assessed with the use of the C statistic.[13] Specifically, C statistic from multivariate models with and without the biomarker of interest are presented and descriptively compared. Also, the net reclassification index, assessing the change in discriminatory ability of the multivariate risk factor model when the biomarker is added, is presented for each biomarker. The net reclassification index is calculated as in Pencina MJ et al.[14] Specifically, subjects were first grouped into the following categories according to the predicted risk calculated from the *non-biomarker* multivariate risk factor model model: 0-2.5%, 2.5%-5%, 5-10%, and >10%. Then, we added the biomarker to the multivariate model and calculated (a) the percentage of subjects *without* an event who moved into a *lower* predicted risk category when the biomarker was added to the model; and (b) the percentage of subjects *with* an event who moved into a *higher* predicted risk category when the biomarker was added to the model. The sum of these two percentages is the net reclassification index (NRI); NRI > 0% for a biomarker that improves the discriminatory ability of the multivariate risk factor model. For each biomarker, a statistical test assessing whether the NRI is 0%, as discussed in Pencina et al., was carried out at the two-sided 0.05 level of significance. A two-sided p-value <0.05 indicates the NRI is significantly different from 0%.

RESULTS

We prospectively studied 3203 subjects who were enrolled in the FHS offspring cohort and participated in examination cycle 5 between 1991-1995. For the assessment of the

association of examination cycle 5 biomarkers with incident CVD, we excluded subjects with prior myocardial infarction, angina pectoris, congestive heart failure, or stroke at cycle 5. For the assessment of the association of PAI-1 change from examination cycle 5 to cycle 6 with incident CVD, we further excluded subjects with prevalent CVD at cycle 6. Follow up from examination cycle 5 was a mean of 10±1.5 years, and from cycle 6, a mean of 6± 1 years. During follow-up from examination 5, 8.9% of men and 3.8% of women had a first CVD event.

The subject characteristics based on quartile of PAI-1 are described in Table 1. The mean age of subjects at baseline (cycle 5) was 54.2 years. Forty-one percent were male, 19% were smokers and 5.4% had diabetes mellitus. Increasing PAI-1 levels were associated with a worse risk factor profile, as indicated by higher age, more men, more diabetes mellitus, higher systolic blood pressure, cigarette smoking, total cholesterol and triglycerides, reduced HDL cholesterol levels, and higher BMI. Similar differences across quartile of TPA were observed (Table 1B). PAI-1 levels were closely correlated with TPA antigen levels (R=0.68, $p<0.001$)

Baseline PAI-1 levels (mean ± SD) were 29.1 ng/ml (19.2) for participants with incident CVD versus 22.1 (16.5) for those without incident CVD; $p<0.001$. TPA levels were 12.0 ng/ml (5.7) versus 9.0 (4.7) for those with and without incident CVD; $p<0.001$.

The probability of a CVD event is presented in Table 2 according to baseline PAI-1 and TPA levels. Data are presented as HRs of each higher quartile versus the first (lowest) quartile. For PAI-1 antigen, strong linear risk gradients were observed for crude HRs and HRs adjusted for age and sex ($p<0.001$). After adjustment for age, sex, smoking, the presence or absence of diabetes mellitus, systolic blood pressure, antihypertensive therapy, BMI, total cholesterol, HDL cholesterol, and triglycerides, the multivariate-adjusted hazard ratios of CVD event for higher quartiles of PAI-1 (the first quartile was the reference) were 1.9, 1.9, 2.6 ($p=0.006$). For TPA antigen, strong linear risk gradients were also observed for crude HRs and HRs adjusted for age and sex ($p<0.001$). After full adjustment, the multivariate hazard ratios of a CVD event for higher quartiles of TPA antigen (the first quartile was the reference) were 1.6, 1.6, 2.9 ($p<0.001$)

The baseline clinical characteristics of the study population according to quartile of change in PAI-1 from baseline to 4 years later shown in Table 3A while in Table 3B, the change in PAI-1 is associated with the change in the clinical characteristics from baseline to 4 years.

Table 3B demonstrates that a reduction in PAI-1 over time in quartile 1 was associated with greater improvements in risk markers and probable lifestyle than seen in the other quartiles. Nonetheless, the quartile with the greatest increase in PAI-1 levels 4 years after baseline had the greatest subsequent CVD event rate after a 6 year mean follow-up after adjustment for either baseline characteristics (Table 4) or serial change in characteristics.

The hazard ratio of CVD for increasing quartile of serial change in PAI-1, adjusted for baseline variables at examination cycle 5, were 0.9, 0.8, 1.3 versus the lowest quartile ($p=0.174$ for linear trend across quartiles: $p=0.050$ for linear trend across continuous change in PAI-1). A similar increase in hazard ratio of CVD was observed for increasing quartile of

serial change in PAI-1 when adjustment was performed for serial change over 4 years in the aforementioned covariates rather than baseline levels, with hazard ratios of 0.9 (0.6-1.4), 1.0 (0.6-1.5) and 1.4 (0.9-2.1) versus the lowest quartile (p=0.072 for linear trend across quartiles; p=0.008 for linear trend across continuous change in PAI-1).

C Statistic and Reclassification

Baseline prediction: The C statistic for the multivariate CVD model from baseline level was 0.805, increasing slightly to 0.808 when PAI-1 was added to the model (net reclassification 4.8%, p = 0.113) and to 0.812 when TPA was added (net reclassification 6.8%, p=0.037). *Serial change of PAI-1:* The C statistic for the multivariate CVD model (including baseline PAI-1 as a risk factor) was 0.691. When adding serial change in PAI-1, the C statistic was 0.696 (net reclassification 2.7% (p=0.417).

DISCUSSION

We observed that PAI-1 levels were an independent predictor of a first CVD event. Since PAI-1 is the major inhibitor of the endogenous fibrinolytic system, this finding suggests that reduced fibrinolytic potential plays an important role in CVD risk.[1] TPA antigen levels, which are closely correlated with PAI-1 levels, were also predictive of a CVD event with a stronger statistical association than seen with PAI-1. Because the PAI-1 concentration in plasma is much higher than TPA antigen concentration, and the TPA antigen assay measures both free and complexed TPA, increased concentration of TPA antigen indicates reduced rather than enhanced fibrinolytic capacity. While TPA antigen has been shown to be an independent predictor in several studies, [15] PAI-1 has not been clearly identified as an independent predictor of a first CVD event.[16] This is the first study to show that serial change in PAI-1 is also related to subsequent CVD risk.

PAI-1 and Baseline Characteristics

Increasing PAI-1 levels were associated with an adverse cardiovascular risk profile. This finding has been observed in previous studies, and includes associations with increasing age and sex, particularly before menopause. [3,15] PAI-1 levels have also been associated with hypertension, diabetes mellitus, triglyceride levels, and homocysteine, and inversely related to HDL cholesterol. PAI-1 is also a component of the insulin resistance syndrome. [17,18]

PAI-1 and Cardiovascular Risk

PAI-1 may increase CVD risk in several ways. As the major inhibitor of the endogenous fibrinolytic system, elevated PAI-1 would reduce the capacity of the fibrinolytic system to prevent fibrin deposition in vessel walls and thrombus formation.[1] PAI-1 is a marker of endothelial injury and may be an intermediary mechanism by which other risk factors injurious to the endothelium exert their effect. Insulin, glucocorticoids, angiotensin II, very low-density lipoprotein, and acute phase cytokines all induce PAI-1 gene transcription.[19] PAI-1 may also impair normal vascular remodeling through effects on integrin expression and cellular migration. [20,21] It remains uncertain how much of the link between PAI-1 and CVD risk is causal versus how much is as a marker of vascular disease, since there is enhanced PAI-1 expression in diseased vessels.[22] Furthermore, while polymorphisms such

as 4G/5G are associated with differences in PAI-1 levels, they have not consistently predicted CVD. [23-25] Our finding that serial change in PAI-1 predicted cardiovascular risk independent of baseline PAI-1 level and changes in other risk markers requires confirmation in other studies. However the finding suggests that progressive change in endothelial dysfunction and prothrombotic state, as indicated by increasing PAI-1 levels, may promote CVD events. The incremental predictive value of serial changes has previously been described for variables such as ECG changes in left ventricular hypertrophy,[26,27] microalbuminuria in resistant hypertension,[28] and the cardio-ankle vascular index for assessment of arterial stiffness.[29]

TPA and Cardiovascular Risk

In the current study, TPA antigen was a significant predictor of CVD events independent of other factors. This finding is consistent with prior studies, although Lowe, in a meta-analysis, noted that the independence of the association was unclear.[8] While elevated TPA antigen levels reflect impaired fibrinolytic potential through its reflection of TPA-PAI-1 complexes, and are inversely related to TPA activity,[30] the assay characteristics of TPA antigen may make it a more robust measure than PAI-1 for multisite epidemiologic studies. In the present study, blood samples were obtained between 8-9 am to minimise circadian effects of PAI-1.[31] Framingham is a single-site study, where laboratory processing and analysis could be readily controlled.

Limitations

The FHS participants in this study are Caucasian, so we cannot be certain that the results would apply to other populations. The average age of participants was 54 years, so we cannot extrapolate to populations of significantly different age. Although we adjusted for conventional risk factors, we cannot exclude that other biomarkers might modify the risk. [32] In particularly, the relationship with fibrinolysis may be attenuated by adjustment for inflammatory markers such as CRP, as has been described. [33] However, Pradham and colleagues noted that elevated TPA antigen was independently associated with incident coronary events, including after adjustment for CRP.[34] C-statistic assessment showed that adding PAI-1 or TPA to conventional risk factors resulted in only modest increases in the ability to classify risk, although more so for TPA. The baseline and 4-year blood samples were analysed at a similar time interval after collection, using the same assay. The samples were therefore not analysed concurrently, although any variability induced would have reduced the likelihood of seeing significant associations.

Conclusions

We have demonstrated, among individuals with no prior CVD, that PAI-1 antigen is a predictor of CVD independent of conventional risk factors. Since elevated PAI-1 levels are associated with a prothrombotic state as well as acute phase reaction, this association supports the importance of thrombosis and inflammation to CVD.[33,34] Elevations of PAI-1 may also be a mechanism by which factors injurious to the endothelium, such as hypertension, diabetes mellitus, cigarette smoking, and hyperlipidemia, increase risk of CVD. In addition, a serial increase in PAI-1 level provides additional information on subsequent risk of CVD. There were only modest increases in the ability to classify risk by

adding PAI-1 to conventional risk factors. TPA antigen had a stronger statistical association with CVD risk, and may be more suitable for epidemiological study. Nonetheless, from a mechanistic perspective, since TPA correlates closely with PAI-antigen, our findings support further investigation of the inhibition of PAI-1 as a potential therapeutic strategy.[35,36]

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Highlights

- PAI-1 and TPA antigen levels predict first CVD events in Framingham Heart Study.
- A serial increase in PAI-1 is associated with an increase in cardiovascular risk.
- These findings support the importance of fibrinolytic potential in CVD.

Table 1A

Characteristics of the study population based on quartile of PAI-1

	Quartile 1 (n=800)	Quartile 2 (n=801)	Quartile 3 (n=801)	Quartile 4 (n=801)	Linear Trend P value
PAI-1 (ng/ml)	7.8 ± 2.3	14.7 ± 1.9	22.9 ±2.9	44.3 ±18.4	
Ln(PAI-l)	2.0 ± 0.4	2.7 ± 0.1	3.1 ± 0.1	3.7 ± 0.3	
Age (years)	52 ± 10	55 ± 10	56 ± 10	56 ± 9	<0.001
Male (%)	30.5	45.7	49.6	55.1	<0.001
Systolic Blood Pressure (mmHg)	117± 17	124 ± 18	128 ± 19	134 ± 17	<0.001
Antihypertensive Therapy (%)	8.4	12.6	18.2	26.7	<0.001
Diabetes Mellitus (%)	1.4	2.9	5.6	15.6	<0.001
Cigarette Smokers (%)	15.0	20.0	19.6	22.0	<0.001
Total cholesterol (mg/dl)	195 ± 34	203 ± 36	209 ± 36	213 ± 38	<0.001
HDL cholesterol (mg/dl)	58.1 ± 15.2	51.7 ± 14.4	48.5 ± 14.6	43.6 ± 12.6	<0.001
Triglycerides (mg/dl)	100 ± 49	124 ± 65	147 ± 75	215 ± 157	<0.001
BMI (m^2/kg)	24.2 ± 3.6	26.4 ± 4.1	27.9 ± 4.6	31.0 ± 5.3	<0.001

Mean ± Standard Deviation

Table 1B

Characteristics of the study population based on quartile of TPA

	Quartile 1 (n=799)	Quartile 2 (n=796)	Quartile 3 (n=797)	Quartile 4 (n=799)	Linear Trend P value
TPA (ng/ml)	4.5 ± 1.1	7.3 ± 0.7	9.8 ±0.8	14.9 ±5.8	
Ln(TPA)	1.5 ± 1.5	2.0 ± 0.1	2.3 ± 0.1	2.7 ± 0.2	
Age (years)	50 ± 9	54 ± 10	56 ± 9	58 ± 9	<0.001
Male (%)	27.7	43.3	51.6	57.8	<0.001
Systolic Blood Pressure (mmHg)	123 ± 17	124 ± 18	130 ± 19	135 ± 18	<0.001
Antihypertensive Therapy (%)	6.0	10.6	21.3	28.0	<0.001
Diabetes Mellitus (%)	6.3	3.5	7.5	14.0	<0.001
Cigarette Smokers (%)	17.5	19.8	16.7	22.1	0.08
Total cholesterol (mg/dl)	192 ± 33	203 ± 36	209 ± 35	215 ± 38	<0.001
HDL cholesterol (mg/dl)	58.7 ± 15.4	51.3 ± 14.1	48.1 ± 14.1	44.0 ± 13.1	<0.001
Triglycerides (mg/dl)	97 ± 45	124 ± 77	158 ± 103	206 ± 138	<0.001
BMI (m^2/kg)	24.5 ± 3.9	26.4 ± 4.2	28.6 ± 4.9	30.0 ± 5.4	<0.001

Mean ± Standard Deviation

Table 2A

Hazard Ratio of a first Cardiovascular Event according to Quartile of PAI-1 Antigen

Quartile of PAI-1 (ng/ml)	**1 (1.2 – 11.4)**	**2 (11.4-18.1)**	**3 (18.2-28.4)**	**4 (28.4-165.2)**	**p-value***	**p-value****
Crude hazard ratio (95% CI)	1.0	3.3 (2.0-5.3)	3.9 (2.4-6.3)	6.4 (4.0-10.1)	<0.001	<0.001
Age and sex-adjusted HR (95% CI)	1.0	2.4 (1.5-3.9)	2.6 (1.6-4.2)	4.3 (2.7-6.9)	<0.001	<0.001
Risk factor-adjusted HR (95% CI)	1.0	1.9 (1.2-3.2)	1.9 (1.2-3.1)	2.6 (1.5-4.3)	<0.001	0.006

Table 2B

Hazard Ratio of a first Cardiovascular Event according to Quartile of TPA antigen (ng/ml)

Quartile of TPA antigen (ng/ml)	**1 (1.2 – 6.1)**	**2 (6.1-8.5)**	**3 (8.5-11.3)**	**4 (11.3-89)**	**p-value***	**p-value****
Crude hazard ratio (95% CI)	1.0	2.7 (1.6-4.5)	3.5 (2.1-5.8)	9.4 (5.9-15.0)	<0.001	<0.001
Age and sex-adjusted HR (95% CI)	1.0	1.9 (1.2-3.3)	2.2 (1.3-3.6)	4.9 (3.0-8.0)	<0.001	<0.001
Risk factor-adjusted HR (95% CI)	1.0	1.6 (1.0-2.8)	1.6 (0.9-2.6)	2.9 (1.7-4.9)	<0.001	<0.001

Risk-factor-adjusted hazard ratios and p-values have been adjusted for age, gender, systolic blood pressure, anti-hypertensive therapy, BMI, diabetes mellitus, cigarette smoking, total cholesterol, HDL cholesterol, and triglycerides. For all hazard ratios risks, the reference is the first quartile.

* P values are for tests of linear trend across biomarker quartiles.

** P-values adjusted for continuous log biomarkers.

Table 3A

Change in PAI-1 after 4 years versus baseline clinical characteristics

	Quartile 1 (n=683)	Quartile 2 (n=684)	Quartile 3 (n=684)	Quartile 4 (n=684)	Linear Trend P value	Quadratic Trend P-Value
Change in PAI-1	−12.6 ± 13.0	0.6 ± 1.7	6.7 ± 2.1	22.4 ± 13.6		
Age (years)	55 ± 9	54 ± 10	55 ± 10	55 ± 10	0.83	0.39
Male (%)	45.9	41.0	45.1	46.6	0.47	0.10
Systolic Blood Pressure (mmHg)	128 ± 19	122 ± 18	124 ± 18	127 ± 18	0.67	<0.001
Antihypertensive Therapy (%)	18.6%	14.3%	13.0%	17.5%	0.48	0.002
Diabetes Mellitus (%)	7.6	3.5	3.6	6.9	0.59	<0.001
Cigarette Smokers (%)	19.9	17.7	15.6	18.2	0.29	0.10
Total cholesterol (mg/dl)	207 ± 38	201 ± 36	204 ± 36	207 ± 35	0.59	0.003
HDL cholesterol (mg/dl)	48.5 ± 14.1	53.4 ± 15.5	53.0 ± 15.8	48.2 ± 13.9	0.62	<0.001
Triglycerides (mg/dl)	163 ± 120	123 ± 79	128 ± 90	156 ± 107	0.35	<0.001
BMI (m^2/kg)	28.2 ± 5.2	26.0 ± 4.4	26.7 ± 4.6	28.5 ± 5.5	0.12	<0.001
Baseline PAI-1 (ng/ml)	34.3 ± 21.5	16.7 ± 10.7	16.7 ± 10.6	20.2 ± 12.3	<0.001	<0.001

Mean ± Standard Deviation

Table 3B

Change in PAI-1 after 4 years versus Serial Change in Clinical Characteristics

	Quartile 1 (n=684)	Quartile 2 (n=685)	Quartile 3 (n=685)	Quartile 4 (n=684)	Linear Trend P value	Quadratic Trend P-Value
Change in PAI-1	−12.6 ± 13.0	0.6 ± 1.7	6.7 ± 2.1	22.4 ± 13.6		
Age (years)	4.0 ± 0.6	4.0 ± 0.5	4.1 ± 0.6	4.1 ± 0.6	0.06	0.68
Male (%)	45.9	41.0	45.1	46.6	0.47	0.10
Systolic Blood Pressure (mmHg)	2 ± 17	3 ± 15	3 ± 15	4 ± 16	0.005	0.81
Antihypertensive Therapy (%)	8.5	7.9	10.4	9.8	0.26	0.99
Diabetes Mellitus (%)	1.9	1.4	2.0	5.1	0.002	0.015
Cigarette Smokers (%)	−3.3	−3.2	−2.4	−0.4	0.69	0.29
Total cholesterol (mg/dl)	−4 ± 31	0.3 ± 28	3 ± 27	6 ± 42	<0.001	0.63
HDL cholesterol (mg/dl)	2.6 ± 9.3	1.7 ± 9.2	0.2 ± 8.8	−0.5 ± 9.0	<0.001	0.87
Triglycerides (mg/dl)	−23 ± 93	−6 ± 62	−3 ± 74	7 ± 218	<0.001	0.51
BMI (m^2/kg)	−0.1 ± 2.1	0.3 ± 1.5	0.7 ± 1.5	1.3 ± 2.3	<0.001	0.48
Baseline PAI-1 (ng/ml)	34.3 ± 21.5	16.7 ± 10.7	16.7 ± 10.6	20.2 ± 12.3	<0.001	<0.001

Mean ± Standard Deviation

Table 4

Hazard Ratio of a first Cardiovascular Event according to the change in PAI-1 from Baseline to 4 Years Later

Quartile of change in PAI-1 (ng/ml)	Quartile 1 (−130.7- −2.6)	Quartile 2 (−2.6 - 3.5)	Quartile 3 (3.5 – 10.8)	Quartile 4 (10.8-102.7	p-value*	p-value**
Crude hazard ratio (HR) (95% CI)	1.0	0.9 (0.6 – 1.4)	1.0 (0.6 – 1.5)	1.4 (0.9 – 2.0)	0.098	0.015
Age & sex-adjusted HR (95% CI)	1.0	0.9 (0.5 – 1.3)	0.9 (0.6 – 1.5)	1.3 (0.9 – 2.0)	0.091	0.006
Risk factor-adjusted HR (95% CI)	1.0	0.9 (0.6 – 1.4)	0.8 (0.6 – 1.5)	1.3 (0.8 – 1.9)	0.174	0.050

Risk-factor-adjusted hazard ratios have been adjusted for gender, and Baseline age, systolic blood pressure, anti-hypertensive therapy, diabetes mellitus, cigarette smoking, total cholesterol, HDL cholesterol, and triglycerides and BMI at exam 5, and baseline PAI-1 level. For all hazard ratios, the reference is the first quartile.

*P-values are for tests of trend across PAI-1 change in quartiles.

**P-values adjusted for continuous change in PAI-1.