Plasma levels of fibrinolytic proteins and the risk of myocardial infarction in men

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Hypofibrinolysis as measured with overall clot lysis assays is associated with risk of arterial thrombosis. Individual components of the fibrinolytic system, however, have not been studied extensively in relation to arterial disease, or results of studies were inconsistent. The relation between plasminogen and α2-antiplasmin levels and cardiovascular risk factors and the association between plasminogen, α2-antiplasmin, tissue-plasminogen activator (t-PA), and plasminogen activator inhibitor-1 (PAI-1) and risk of myocardial infarction was investigated in the Study of Myocardial Infarctions Leiden (555 men with a first myocardial infarction and 635 controls). α2-antiplasmin was associated with age and lipid levels, whereas plasminogen correlated with lipids, C-reactive protein, and smoking. Increased levels of all fibrinolytic factors were associated with myocardial infarction. Age-adjusted odds ratios (ORs; 95% confidence interval) for quartile 4 compared with 1 were 1.7 (1.2-2.3) for plasminogen, 1.9 (1.3-2.6) for α2-antiplasmin, 1.7 (1.2-2.3) for t-PA, and 1.7 (1.2-2.4) for PAI-1. After adjusting for cardiovascular risk factors, only α2-antiplasmin levels remained associated with risk (OR, 1.4; [1.0-2.0]). t-PA and PAI-1 levels predominantly reflected lipid levels, whereas plasminogen reflected the inflammatory state. Concluding, elevated α2-antiplasmin levels are independently associated with risk of myocardial infarction. t-PA, PAI-1, and plasminogen levels appear to reflect other cardiovascular risk factors. (*Blood*. 2010;116(4):529-536)

Introduction

Decreased fibrinolytic potential as measured with overall clot lysis assays has been found to be associated with increased risk of arterial thrombosis, especially in young persons, in several studies.[1-3] Surprisingly, plasma levels of individual components of the fibrinolytic system have either not been studied extensively in the context of arterial thrombosis or were not consistently associated with arterial thrombosis. In particular, population-based studies on the role of α2-antiplasmin and plasminogen in the risk of arterial thrombosis are scarce. In the European Concerted Action on Thrombosis and Disabilities (ECAT) study, a cohort study, including patients with angina pectoris, no association was found between levels of α2-antiplasmin and risk of myocardial infarction or sudden death.[4] Unexpectedly, increased levels of plasminogen were associated with an increased risk. In the Atherosclerosis Risk in Communities (ARIC) study, a population-based cohort study on subjects between 44 and 65 years of age at baseline, a positive association between plasminogen levels and coronary heart disease was also found.[5]

The results of studies on levels of tissue type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) have been conflicting. Both t-PA and PAI-1 levels were associated with arterial disease in multiple studies. However, whether increased levels of t-PA and PAI-1 independently increase the risk remained to be elucidated.[6] PAI-1 is now recognized as a true component of the metabolic syndrome,[7] which is strongly associated with arterial thrombosis. In several studies the predictive value of PAI-1 and t-PA disappeared after adjusting for cardiovascular risk factors such as body mass index (BMI), insulin resistance, inflammation, and lipid levels (reviewed in Meltzer et al[6]). This may indicate that levels of these fibrinolytic factors are rather a reflection of underlying disease than a direct cause of arterial thrombosis.

Results of studies on the association of plasma levels of thrombin activatable fibrinolysis inhibitor (TAFI) with the risk of arterial thrombosis are also contradictory. Several studies have found increased levels of TAFI to be associated with an increased risk of arterial disease,[8,9] whereas others found no association.[10] We have recently shown increased functional TAFI levels to be associated with a decreased risk of myocardial infarction in the Study of Myocardial Infarctions LEiden (SMILE), a large case-control study in men.[11] In the present study, we investigate associations between levels of plasminogen, α2-antiplasmin, PAI-1, and t-PA and the risk of myocardial infarction in the SMILE. Furthermore, because knowledge on determinants of plasma levels of α2-antiplasmin and plasminogen is scarce, the association between established cardiovascular risk factors and these 2 fibrinolytic factors was also studied.

Methods

Subjects

The design of the SMILE has been described previously.[12] Patients were 560 men with a first myocardial infarction between 1990 and 1996, younger than the age of 70 years at the onset of myocardial infarction. Two of the following 3 characteristics had to be identifiable in the discharge report or hospital care record to confirm acute myocardial infarction: typical chest pain, electrocardiographic changes indicative of evolving myocardial

infarction, or a transient rise in cardiac enzymes to more than twice the upper limit of normal.

The control group consisted of 646 men without a history of myocardial infarction. They had undergone a minor orthopedic intervention between January 1990 and May 1996 for which they had received prophylactic anticoagulants for a short period. They had not received anticoagulants in the 6 months before participation in the study. Control subjects were frequency matched on 10-year age groups to the patients. Every participant completed a questionnaire on cardiovascular risk factors, including questions on current and former smoking habits and alcohol use, presence of diabetes, and current use of medication. In addition, for patients, presence of diabetes before myocardial infarction was retrieved from discharge letters. A person was classified as hypertensive or hypercholesterolemic when he was taking prescription drugs for these conditions. Blood pressure was measured after a rest of at least 10 minutes with the person sitting in an upright position. BMI was derived by dividing weight (in kilograms) by squared height (in meters). All participants gave informed consent. Approval for this study was obtained from the Medical Ethics Committee of the Leiden University Medical Center, Leiden, The Netherlands.

Laboratory analysis

Fasting blood samples of patients and control subjects were drawn from the antecubital vein in Sarstedt Monovette tubes and were obtained between July 1994 and February 1997. Blood samples were primarily drawn in the morning (median, 9:30 AM, 95% before 11:00 AM), without a systematic difference between patients and control subjects. Time between myocardial infarction and blood draw ranged from 88 days to 6 years with a median of 2.6 years.

Serum and plasma samples were divided into aliquots in multiple tubes and were immediately stored at $-80°C$. Plasma levels of von Willebrand factor (VWF) and C-reactive protein (CRP) and serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were measured as described previously.[12,13]

Plasma levels of fibrinolytic factors were measured in citrated plasma. $\alpha2$-antiplasmin and plasminogen activity was measured with the use of chromogenic assays (STA Stachrom antiplasmin and STA Stachrom plasminogen from Diagnostica Stago) and were performed on a STA-R coagulation analyzer with the use of a commercial calibration standard (Diagnostica Stago) and expressed as a percentage of normal. PAI-1 antigen levels were measured with a Technozym PAI-1 enzyme-linked immunosorbent assay reagent kit (Kordia) and were expressed in ng/mL. Antigen levels of t-PA were assessed by enzyme-linked immunoabsorbent assay with the use of a commercially available mouse anti–t-PA antibody (Nuclilab BV) as capture, and a biotin-labeled rabbit anti–human t-PA antibody (Nuclilab BV) as detecting antibody. Bound detecting antibody was visualized with the use of biotin-labeled streptavidin, followed by tetramethylbenzidine staining. A calibration curve was constructed with the use of purified t-PA (Nuclilab BV), and the results were expressed as ng/mL.

The intraassay coefficients of variation were 1.7% for plasminogen, 4.8% for $\alpha2$-antiplasmin, 7.6% for PAI-1, and 11.4% for t-PA, and the interassay coefficients of variation were 1.6% for plasminogen, 4.6% for $\alpha2$-antiplasmin, 5.0% for PAI-1, and 8.1% for t-PA.

In 5 patients and 11 control subjects, the fibrinolytic protein levels were not measured because available plasma was not sufficient, leaving 555 patients and 635 control subjects in the analyses.

Statistical analysis

The association between cardiovascular risk factors and plasma levels of $\alpha2$-antiplasmin and plasminogen were studied in the control group. Mean $\alpha2$-antiplasmin and plasminogen levels were calculated with 5th and 95th percentiles for categories of cardiovascular risk factors. Quartiles of blood pressure, total cholesterol, HDL cholesterol, triglyceride, VWF, and CRP were defined according to the distribution among control subjects. Multiple linear regression was used to investigate which factors were independently associated with levels of $\alpha2$-antiplasmin and plasminogen. Because the associations between triglycerides, VWF, and CRP with $\alpha2$-antiplasmin

and plasminogen were not linear, these variables were entered in the model divided into quartiles, resulting in a regression coefficient for a quartile increase of the independent variable. For reasons of comparability systolic blood pressure, HDL cholesterol, and total cholesterol were also included in the model as quartiles as were levels of $\alpha2$-antiplasmin when studied as determinants of plasminogen and vice versa. Using 10log-transformation for the not normally distributed variables instead of quartiles did not considerably change the results. However, because a regression coefficient of a 10log-transformed variable is more difficult to interpret than a regression coefficient, for each quartile increase we present the latter.

To study the effect of fibrinolytic factors on risk of myocardial infarction, levels of $\alpha2$-antiplasmin, plasminogen, PAI-1, and t-PA were grouped into quartiles according to the distribution among the control subjects, taking the lowest quartile as the reference group for the odds ratio (OR). A 95% confidence interval (CI) was calculated according to the method of Woolf.[14] Unconditional logistic regression was performed to adjust for age and other potential confounders (see "Confounders"). In the logistic regression model age, BMI, blood pressure, VWF, CRP, and lipid levels were included as continuous variables. Triglycerides, VWF, and CRP levels were included in the model after 10log-transformation because these variables were not normally distributed. Subgroups were made according to age. An arbitrary cutoff at age 50 years was chosen in accordance to previous publications on the SMILE.[1,15,16] SPSS 16.0 (SPSS Inc) was used for statistical analyses.

Confounders

Available literature on factors that influence plasma levels of $\alpha2$-antiplasmin and plasminogen is limited. Associations between established cardiovascular risk factors and levels of $\alpha2$-antiplasmin or plasminogen were studied in our own data, and potential confounders were selected from these analyses and included in the statistical model.

Several studies investigated determinants of t-PA and PAI-1. Therefore, confounding variables in the association between t-PA or PAI-1 and myocardial infarction were chosen from these studies and included in the models, ie, diabetes, BMI, lipid levels, plasma levels of VWF and CRP, and blood pressure.[4,7] Indeed, these factors were also associated with t-PA and PAI-1 levels in our own data, with the exception of VWF, which was not associated with PAI-1 in our data (data not shown). The analyses on PAI-1 and t-PA and risk of myocardial infarction were also mutually adjusted for each other.

Results

Mean age of the 555 patients with myocardial infarction was 56.3 years (5th-95th percentiles, 40.0-68.8 years) and mean age of 635 control subjects was 57.4 years (5th-95th percentiles, 34.7-72.1 years). Risk factors for arterial disease such as smoking, obesity, diabetes, hypertension, and hypercholesterolemia were more prevalent in patients than in control subjects (Table 1).

Cardiovascular risk factors and plasma levels of $\alpha2$-antiplasmin and plasminogen

The association between risk factors for myocardial infarction and $\alpha2$-antiplasmin levels in control subjects is shown in Table 2. $\alpha2$-antiplasmin was negatively associated with age, HDL cholesterol, VWF, and systolic blood pressure level and was positively associated with total cholesterol, triglyceride, and plasminogen levels. $\alpha2$-antiplasmin also increased with BMI, although the small group of men (n = 10) with BMI less than 20 kg/m^2 did not have low levels. To determine the independent effect of these factors on $\alpha2$-antiplasmin levels, age, BMI, HDL cholesterol, VWF, systolic blood pressure, total cholesterol, triglyceride, and plasminogen were simultaneously included in a multiple linear regression

Table 1. Characteristics of men with first myocardial infarction and control subjects

Characteristic	Patients (n = 555)	Control subjects (n = 635)
Mean age, y (5th-95th percentile)	56.3 (40.0-68.8)	57.4 (34.7-72.1)
Current smoking, no. (%)		
No	208 (37.5)	426 (67.1)
Yes	347 (62.5)	209 (32.9)
Alcohol use, no. (%)		
Never	86 (15.5)	64 (10.1)
Occasionally	24 (4.3)	20 (3.1)
Regularly	445 (80.2)	551 (86.8)
BMI, kg/m², no. (%)*		
Less than 20	4 (0.7)	10 (1.6)
20-24	152 (27.4)	182 (28.7)
25-30	305 (55.1)	341 (53.8)
At least 30	93 (16.8)	101 (15.9)
Diabetes, no. (%)		
Absent	529 (95.3)	614 (96.7)
Present	26 (4.7)	21 (3.3)
Hypertension, no. (%)		
Absent	449 (80.9)	529 (83.3)
Present	106 (19.1)	106 (16.7)
Hypercholesterolemia, no. (%)		
Absent	544 (98.0)	623 (98.1)
Present	11 (2.0)	12 (1.9)

Data for patients refer to the period before myocardial infarction, apart from BMI.
*BMI was missing for 1 patient and 1 control subject.

model. Age ($\beta = -0.3\%$/year; 95% CI, -0.4 to -0.2) and HDL cholesterol ($\beta = -1.2$; 95% CI, -2.2 to -0.3) were both negatively associated with α2-antiplasmin. BMI ($\beta = 0.3\%$/(kg/m²); 95% CI, 0.0-0.6) and total cholesterol (0.8%/quartile; 95% CI, -0.1 to 1.8) were positively associated with α2-antiplasmin. Plasminogen was strongly related to α2-antiplasmin ($\beta = 2.5\%$/quartile; 95% CI, 1.8-3.4). Systolic blood pressure ($\beta = -0.5\%$/quartile; 95% CI, -1.5 to 0.4), triglycerides ($\beta = -0.2\%$/quartile; 95% CI, -1.3 to 0.8), and VWF ($\beta = 0.0\%$/quartile) were not associated with α2-antiplasmin. Therefore, HDL cholesterol, total cholesterol, and plasminogen were the strongest determinants of α2-antiplasmin.

Plasminogen levels increased with levels of triglycerides, total cholesterol, and CRP and was increased in smokers (Table 2). In addition, alcohol use was associated with plasminogen, although not in a dose-dependent manner because the occasional drinkers had the lowest levels of plasminogen. Similar to α2-antiplasmin, plasminogen increased with BMI, but the small group of underweight subjects (BMI < 20 kg/m²) had high levels. We performed a multiple regression analysis that included age, BMI, triglycerides, total cholesterol, CRP, alcohol use, smoking, and α2-antiplasmin levels. Except for age, all variables were independently associated with plasminogen. The regression coefficients were 1.6%/quartile (95% CI, 0.9-2.4) for total cholesterol and 1.2%/quartile (95% CI, 0.2-1.9) for triglycerides. Plasminogen increased 2.9% (95% CI, 2.2%-3.6%) with each quartile increase of CRP and 2.2% (95% CI, 1.5%-2.9%) per quartile increase in α2-antiplasmin. Compared with occasional drinkers of alcohol, regular drinkers had 5.7% higher levels (95% CI, 1.5%-10.0%), and abstainers had 3.4% higher levels (95% CI, -1.4% to 8.2%). Smoking increased plasminogen levels with 3.6% (95% CI, 1.9%-5.2%) compared with not smoking. Therefore, plasminogen was strongly associated with variables related to inflammation.

Plasma levels of fibrinolytic proteins and risk of myocardial infarction

α2-antiplasmin. Mean α2-antiplasmin level in patients was 99% (median, 98%; 5th-95th percentile, 81%-119%) and 96% in controls (median, 95%; 5th-95th percentile, 77%-116%). Levels of α2-antiplasmin were associated with risk of myocardial infarction in a dose-dependent manner. In men with the highest levels of α2-antiplasmin, the age-adjusted risk was approximately 2-fold increased (model 1: OR, 1.9; 95% CI, 1.3-2.6; 4th quartile [Q] compared with first Q; Table 3). Because lipid levels were the strongest determinants of α2-antiplasmin levels, apart from plasminogen levels, we first adjusted for HDL and total cholesterol (model 2). This reduced the OR, but high levels of α2-antiplasmin were still associated with an increased risk of myocardial infarction (OR, 1.5; 95% CI, 1.0-2.1). Further adjustment for BMI (model 3: OR, 1.5; 95% CI, 1.1-2.2) and additional adjustment for plasminogen did not reduce the OR further (model 4: OR, 1.4; 95% CI, 1.0-2.0). The same analysis in men younger than 50 years resulted in an age-adjusted OR of 2.6 (95% CI, 1.2-5.9; Q4 vs Q1) and 1.6 (95% CI, 0.7-3.8) after adjusting for age, HDL and total cholesterol, BMI, and plasminogen (model 4). In men older than 50 years, these age-adjusted ORs were 1.7 (95% CI, 1.2-1.7) and 1.4 (95% CI, 0.9-2.2) after extensive adjustment.

Plasminogen. Mean plasminogen level in patients was 96% (median, 96%; 5th-95th percentile, 79%-115%) and 94% in controls (median, 94%; 5th-95th percentile, 77%-113%). The risk of myocardial infarction increased with each increasing quartile of plasminogen (Table 4). The risk in men with the highest levels of plasminogen was 1.7-fold (95% CI, 1.2-2.3; Q4 vs Q1) increased after adjusting for age (model 1). Because plasminogen levels were strongly associated with variables related to inflammation, we adjusted for CRP and smoking (model 2), reducing the OR to no effect (OR, 1.1; 95% CI, 0.7-1.5). Adjusting for age and CRP or age and smoking separately yielded similar results as did simultaneous adjustment for age and both CRP and smoking (data not shown). Adding triglycerides, total cholesterol, and alcohol use marginally changed the OR (0.9; 95% CI, 0.06-1.3; model 3) as did additional adjustment for α2-antiplasmin levels (OR, 0.8; 95% CI, 0.5-1.2; model 4).

Similar results were found when analyses were performed separately in men younger than 50 years and in men 50 years and older. Although the age-adjusted risk in young men was higher (OR, 2.6; 95% CI, 1.3-5.0; Q4 vs Q1) than in the older men (OR, 1.3; 95% CI, 0.9-1.9; Q4 vs Q1), the increased risks disappeared after further adjustment for smoking, alcohol use, and levels of triglycerides, total cholesterol, and α2-antiplasmin (model 4; OR, 0.8; 95% CI, 0.3-1.8 in men < 50 years of age and OR, 0.7; 95% CI, 0.4-1.1 in men > 50 years).

PAI-1. Mean PAI-1 level in patients was 107.4 ng/mL (median, 69.7 ng/mL; 5th-95th percentile, 14.7-316.9 ng/mL) and 88.8 ng/mL in controls (median, 54.9 ng/mL; 5th-95th percentile, 13.0-302.7 ng/mL). Those men with high PAI-1 levels had an increased risk of myocardial infarction (OR, 1.7; 95% CI, 1.2-2.3; Q4 vs Q1), but no dose-response relation was found after adjusting for age (model 1; Table 5). Because PAI-1 is a marker of the insulin resistance syndrome, we first adjusted for triglycerides, HDL and total cholesterol, BMI, and diabetes (model 2), resulting in an OR of 1.1 (95% CI, 0.8-1.6), with the largest effect after adjusting for triglycerides and HDL cholesterol (data not shown). Adjusting only for age and CRP to determine the role of inflammation in the association between PAI-1 and myocardial infarction only slightly decreased the OR to 1.5 (95% 1.1-2.1; Q4 vs Q1). Further

Table 2. Cardiovascular risk factors among 635 control subjects and the association with α2-antiplasmin and plasminogen levels

Characteristic	n	α2-Antiplasmin level		Plasminogen level	
		Mean	5th-95th percentile	Mean	5th-95th percentile
Age, y					
27-39	54	104	84-122	93	74-107
40-49	103	98	80-121	94	77-113
50-59	178*	97	77-115	97	78-114
60-69	243	93	74-114	94	77-114
70-75	57	90	78-108	91	72-115
BMI, kg/m²†					
Less than 20	10	97	74-115	99	79-115
20-24	182	94	74-112	92	72-112
25-29	341	96	78-116	94	78-112
At least 30	101*	98	78-123	98	80-116
Diabetes					
No	614*	96	77-116	94	77-113
Yes	21	95	78-123	92	70-113
Diastolic blood pressure, mm Hg‡					
Less than 80	199	96	76-119	93	77-115
85	90*	96	81-116	96	76-114
90	167	94	74-114	93	74-114
At least 95	174	97	78-116	96	78-112
Systolic blood pressuremm Hg‡					
Less than 125	157	98	75-121	94	76-113
130-135	114	95	77-116	94	80-109
140-150	200*	96	78-115	94	75-115
At least 155	159	93	78-112	95	76-115
Triglycerides, mmol/L†					
Less than 0.90	157	94	74-115	90	72-109
0.90-1.24	159	95	76-113	94	76-111
1.25-1.82	161	96	78-117	96	80-115
At least 1.83	157	98	81-118	98	81-116
Total cholesterol, mmol/L†					
Less than 5.17	161	94	75-113	91	72-111
5.17-5.81	156	95	76-117	93	76-111
5.82-6.59	157	97	80-119	94	79-110
At least 6.60	160	98	80-118	99	84-116
HDL cholesterol, mmol/L§					
Less than 1.1	159	98	78-116	95	76-116
1.1-1.28	156	97	80-119	94	76-113
1.29-1.53	162	94	73-114	94	76-112
At least 1.54	155	95	78-117	94	78-115
C-reactive protein, mg/L					
Less than 0.78	159	95	74-113	89	74-108
0.78-1.55	161	96	78-116	93	78-107
1.56-3.42	159*	96	78-119	95	76-119
At least 3.43	156	96	78-116	101	85-118
Smoking					
No	426*	96	78-117	93	76-112
Yes	209	95	75-115	98	80-116
Alcohol use					
No	64	96	79-117	92	74-111
Occasionally	20	95	55-123	89	70-106
Yes	551*	96	77-116	95	77-114
VWF‖					
Less than 97%	159	97	77-117	92	74-108
98%-124%	151	97	81-118	94	78-114
125%-158%	145	96	74-114	96	77-115
At least 159%	152	93	75-114	96	77-117
Plasminogen*					
Less than 87%	171	91	74-109		
87%-93%	152	95	76-114		
94%-100%	155	97	81-116		
At least 101%	156	100	81-120		
α2-antiplasmin					
Less than 87%	163			91	72-109
87%-94%	163			93	76-112
95%-104%	153			95	76-113
At least 105%	155			99	84v116

BMI indicates body mass index; HDL, high-density lipoprotein; and VWF, von Willebrand factor.

*Plasminogen levels were missing for 1 control subject.

†BMI was missing for 1 control subject.

‡Systolic and diastolic blood pressures were missing for 5 control subjects.

§HDL cholesterol was missing for 3 control subjects.

‖VWF was missing for 28 control subjects.

Table 3. Risk of myocardial infarction with increasing quartiles of α2-antiplasmin

	Q1	Q2	Q3	Q4
Cutoff, %	86	93	100	
No. of patients	96	128	152	178
No of control subjects	163	163	154	155
Model 1: age, OR (95% CI)	1	1.3 (0.9-1.9)	1.6 (1.2-2.3)	1.9 (1.3-2.6)
Model 2: model 1 + HDL and total cholesterol, OR (95% CI)	1	1.2 (0.8-1.7)	1.5 (1.0-2.1)	1.5 (1.0-2.2)
Model 3: model 2 + BMI, OR (95% CI)	1	1.2 (0.8-1.7)	1.5 (1.0-2.1)	1.5 (1.0-2.2)
Model 4: model 3 + plasminogen, OR (95% CI)	1	1.2 (0.8-1.7)	1.4 (1.0-2.0)	1.4 (1.0-2.0)

α2-antiplasmin levels were missing for 1 patient.
Q indicates quartile; HDL, high-density lipoprotein; and BMI, body mass index.

adjustments for other potential confounders (CRP, VWF, systolic and diastolic blood pressures; model 3) did not change the results, neither did the additional adjustment for t-PA (model 4). Analyzing men older and younger than 50 years separately gave similar results (data not shown).

t-PA. Mean t-PA level in patients was 9.2 ng/mL (median, 8.9 ng/mL; 5th-95th percentile, 5.0-14.6 ng/mL) and 8.8 ng/mL in controls (median, 8.1 ng/mL; 5th-95th percentile, 4.5-15.3 ng/mL).

The risk of myocardial infarction was increased in men with t-PA levels above the median (age-adjusted OR, 2.0; 95% CI, 1.4-2.7 [Q3]; OR, 1.7; 95% CI, 1.2-2.4) [Q4 vs Q1]; Table 6). Additional adjusting for lipid levels, diabetes, and BMI (model 2) attenuated the risk (OR, 1.5; 95% CI, 1.1-2.2 [Q3]; OR, 1.1; 95% CI, 0.8-1.6 [Q4]), again with the largest reduction in risk after adjustment for triglycerides or HDL cholesterol (data not shown). In contrast, adjusting for age and CRP had only little effect on risk, reducing the OR for Q3 to 1.8 (95% 1.3-2.6) and Q4 to 1.6 (95% CI, 1.1-2.2), and similar results were found after adjusting for age and VWF as marker of endothelial activation (OR, 1.9; 95% CI, 1.3-2.6 [Q3]; and OR, 1.6; 95% CI, 1.2-2.3 [Q4]). Including all potential confounders (lipid levels, diabetes, BMI, VWF, CRP, and systolic and diastolic blood pressures) reduced the risk slightly more (OR, 1.4; 95% CI, 1.0-2.0 [Q3]; OR, 1.0; 95% CI, 0.7-1.5 [Q4] model 3). Adding PAI-1 to the statistical model did not influence the risk (model 4). Results were similar in men of 50 years and older (data not shown). In younger men the age-adjusted risk was slightly decreased in Q2 (OR, 0.8; 95% CI, 0.4-1.4) and greater than 2-fold increased in the upper 2 quartiles (OR, 2.5; 95% CI, 1.3-1.6 [Q3]; OR, 2.8; 95% CI, 1.4-5.4 [Q4]). These ORs decreased to 0.5 (95% CI, 0.2-1.0) for Q2, 1.7 (95% CI, 0.8-3.4) for Q3, and 1.6 (95% CI, 0.7-3.6) when using multivariate model 4.

Discussion

In this study we have shown that increased levels of α2-antiplasmin are associated with a 2-fold increased risk of a first myocardial infarction in men. After adjusting for several potential confounders,

the risk was still 40% increased compared with men with low levels of α2-antiplasmin. Risk of myocardial infarction was also increased in men with elevated levels of plasminogen, PAI-1, and t-PA in age-adjusted models, but this increased risk was not independent of other risk factors.

The present study is the first to show increased α2-antiplasmin levels to be associated with an increased risk of myocardial infarction. This is consistent with established findings on the association between hypofibrinolysis as measured with overall clot lysis assays and an increased risk of arterial thrombosis[1,2] and with the bleeding tendency observed in patients with α2-antiplasmin deficiency.[17] The ECAT study is the only other large study that examined the relation between α2-antiplasmin levels and risk of arterial disease.[4] In this cohort study, which consisted of approximately 2600 patients with angina pectoris at baseline and 97 events after 2 years of follow-up, no association between α2-antiplasmin levels and myocardial infarction and cardiovascular death was found. This difference in study population may explain the divergent results in the ECAT study and the study presented in this article.

Epidemiologic literature on α2-antiplasmin in relation to risk factors for arterial thrombosis is limited. Here, we show that plasma levels of α2-antiplasmin are only marginally influenced by established cardiovascular risk factors. Although levels of most hemostatic factors increase with age, α2-antiplasmin levels decreased with age, which is in agreement with results of the ECAT study.[18] There was also a weak negative association between α2-antiplasmin and HDL cholesterol and a positive association with BMI and total cholesterol. Although the association between lipid levels and α2-antiplasmin has been found before,[19] to our knowledge studies on the mechanism linking lipid levels to levels of α2-antiplasmin are at present lacking. There is no obvious explanation for the strong correlation between plasminogen and α2-antiplasmin plasma levels. It is, however, a common finding that several coagulation and fibrinolytic factors cluster together and correlate possibly through a common regulatory mechanism.[20-22]

Table 4. Risk of myocardial infarction with increasing quartiles of plasminogen

	Q1	Q2	Q3	Q4
Cutoff, %	86	94	104	
No. of patients	112	125	144	174
No. of control subjects	171	152	155	156
Model 1: age, OR (95% CI)	1	1.2 (0.9-1.7)	1.4 (1.0-1.9)	1.7 (1.2-2.3)
Model 2: model 1 + CRP and smoking, OR (95% CI)	1	1.0 (0.7-1.5)	1.1 (0.8-1.5)	1.1 (0.7-1.5)
Model 3: model 2 + triglycerides, total cholesterol, and alcohol use, OR (95% CI)	1	1.0 (0.7-1.4)	1.0 (0.7-1.5)	0.9 (0.6-1.3)
Model 4: model 3 + α2-antiplasmin, OR (95% CI)	1	0.9 (0.6-1.3)	0.9 (0.6-1.3)	0.8 (0.5-1.2)

Plasminogen levels were missing for 1 control subject.
Q indicates quartile; and CRP, C-reactive protein.

Table 5. Risk of myocardial infarction with increasing quartiles of PAI-1

	Q1	Q2	Q3	Q4
Cutoff, ng/mL	32.9	54.9	99.0	
No. of patients	118	109	121	202
No. of control subjects	158	159	159	158
Model 1: age, OR (95% CI)	1	0.9 (0.7-1.3)	1.0 (0.7-1.4)	1.7 (1.2-2.3)
Model 2: model 1 + HDL and total cholesterol, triglycerides, BMI, and diabetes, OR (95% CI)	1	0.8 (0.6-1.2)	0.8 (0.6-1.2)	1.1 (0.8-1.6)
Model 3: model 2 + CRP, VWF, systolic and diastolic blood pressures, OR (95% CI)	1	0.8 (0.6-1.2)	0.8 (0.6-1.2)	1.1 (0.8-1.7)
Model 4: model 3 + t-PA, OR (95% CI)	1	0.8 (0.6-1.2)	0.8 (0.6-1.2)	1.1 (0.8-1.7)

PAI-1 levels were missing for 1 control subject and 5 patients.

PAI-1 indicates plasminogen activator inhibitor 1; Q, quartile, HDL, high-density lipoprotein; BMI, body mass index; CRP, C-reactive protein; VWF, von Willebrand factor; and t-PA, tissue plasminogen activator.

We found elevated plasminogen levels to be associated with risk of myocardial infarction, although the risk disappeared after adjustment for potential confounders. The ECAT[4] and ARIC[5] studies also found a positive association between plasminogen levels and risk of arterial disease, which is contradictory considering the role of plasminogen in fibrinolysis. In the present study the association disappeared after adjusting for lipid levels, CRP, smoking, and alcohol use. In the ECAT study no adjustments were made apart from study center, age, and sex, and the risk of myocardial infarction or sudden death remained only slightly increased after these adjustments.[4] In the ARIC study adjustments were made for several cardiovascular risk factors, although not for triglycerides and CRP, and substantially increased risks were found even after these adjustments.[5] The differences in results between studies may thus be explained by differences in confounding factors considered in the analyses.

We found that regular drinkers of alcoholic beverages had increased plasminogen levels. In a study on hepatocyte cell lines it has indeed been shown that alcohol increases plasminogen gene expression, and a moderate dose of alcohol also increased plasmin levels in mice.[23] Similar to α2-antiplasmin, the association between plasminogen and lipid levels was shown previously, but the mechanism behind the association has not been described.[5] In contrast, various pathways relating plasminogen to inflammation have been described. Elevated plasma levels of plasminogen may reflect an increased inflammatory state as plasminogen transcription is increased by interleukin-6.[24] This may also explain the association between plasminogen and smoking, because smoking leads to an increased inflammatory state.[25] Conversely, there is evidence that plasminogen induces an inflammatory response. When bound to several plasminogen receptors, shown for example for the receptors enolase-1 and histone H2B, plasminogen facilitates transmigration and basement membrane degradation and aids in the degradation of the extracellular matrix and recruitment of macrophages.[26] Thus, increased inflammation can cause increased plasminogen levels and, vice versa, increased plasminogen levels

can increase the inflammatory state. Consequently, as inflammation increases the risk of myocardial infarction,[24,26] the positive association between plasminogen and myocardial infarction can be either indirect and just reflecting the inflammatory state or it can indeed be causal. However, after adjusting for smoking alone, which, in contrast to high CRP levels, can only cause and not result from an increased inflammatory state or high plasminogen levels, we find that the association between plasminogen and myocardial infarction disappears. This provides evidence against a causal role for plasminogen in myocardial infarction.

Both t-PA and PAI-1 were not independently associated with myocardial infarction in our study. Previous studies on t-PA and PAI-1 levels gave conflicting results. Although some studies have found increased t-PA and PAI-1 levels to be associated with an increased risk of arterial disease,[27-29] several studies have found no association,[5,30,31] and in 2 studies even a trend toward a decreased risk was found in subjects with elevated PAI-1.[30,32] These inconsistent results have been ascribed to the adjustments made for confounders in the analyses because these vary across studies.[4,6] In the ECAT study, the prognostic value of t-PA and PAI-1 was studied after adjustment for clusters of confounding variables.[4] Our results on t-PA and PAI-1 are to a large extent in agreement with that study. In the ECAT study the risk of arterial disease associated with increased PAI-1 levels disappeared after adjustment for parameters associated with insulin resistance (BMI, triglycerides, HDL cholesterol, systolic blood pressure, diabetes). In our study these factors, and particularly lipid levels, could also explain the association between PAI-1 and myocardial infarction. In accordance with results of the ECAT study, the effect of t-PA on arterial disease risk was explained by the combination of the markers of insulin resistance, and, although to a lesser extent, to CRP as marker of inflammation and VWF as indicator of endothelial activation. Our results are also, at least in part, in agreement with 2 meta-analyses conducted on the association between t-PA and PAI-1 and coronary heart disease.[33] PAI-1 was not associated with coronary heart disease in this meta-analysis that included 6 prospective cohort

Table 6. Risk of myocardial infarction with increasing quartiles of t-PA

	Q1	Q2	Q3	Q4
Cutoff, ng/mL	6.3	8.1	10.6	
No. of patients	104	104	185	160
No. of control subjects	158	159	159	158
Model 1: age, OR (95% CI)	1	1.1 (0.8-1.5)	2.0 (1.4-2.7)	1.7 (1.2-2.4)
Model 2: model 1 + HDL and total cholesterol, triglycerides, BMI, and diabetes, OR (95% CI)	1	0.9 (0.6-1.3)	1.5 (1.1-2.2)	1.1 (0.8-1.6)
Model 3: model 2 + CRP, VWF, systolic and diastolic blood pressures, OR (95% CI)	1	0.9 (0.6-1.4)	1.4 (1.0-2.0)	1.0 (0.7-1.5)
Model 4: model 3 + PAI-1	1	0.9 (0.6-1.3)	1.4 (1.0-2.0)	1.0 (0.6-1.5)

t-PA levels were missing for 1 control subject and 2 patients.

t-PA indicates tissue plasminogen activator; Q, quartile; HDL, high-density lipoprotein; BMI, body mass index; CRP, C-reactive protein; VWF, von Willebrand factor; PAI-1, plasminogen activator inhibitor 1.

studies. In the meta-analysis that included 12 prospective cohort studies on t-PA and coronary heart disease, an OR of 1.48 was found for the third tertile of t-PA compared with the first. There was, however, evidence of publication bias, showing a tendency to more extreme ORs in the smaller studies, and no adjustments were made for inflammation or VWF, suggesting that this risk estimate was overestimated.

Besides differences in statistical models used, the roles of the fibrinolytic proteins other than in clot dissolution may also have attributed to the inconsistency of results of different studies (reviewed in Meltzer et al[6]). Besides its role in clot lysis, plasminogen possibly contributes to destabilization of atherosclerotic plaques independent of fibrin proteolysis. Plasmin activates several matrix metalloproteinases,[34] and it has been consistently shown that matrix metalloproteinases increase atherosclerosis progression and plaque instability (reviewed by Newby[35]).

Furthermore, PAI-1 can both promote and suppress vascular remodeling by mechanisms not directly related to clot lysis. Local PAI-1 levels have been shown to be associated with severity of atherosclerosis,[36] and PAI-1–deficient mice were found to be protected against atherosclerosis progression in the carotid artery.[37] In contrast, PAI-1 may also protect against abnormal matrix remodeling in advanced atherosclerotic plaques as well as plaque rupture and destabilization of a plaque caused by urokinase plasminogen activator.[38,39] Consequently, heterogeneity in the atherogenic state within and between study populations may hamper direct comparison of study results. Future research may gain more insight in the role of fibrinolytic factors in arterial disease by taking this into account.

Although decreased overall fibrinolytic potential as measured with an overall plasma clot lysis assay has been shown to be associated with an increased risk of myocardial infarction in the SMILE,[1] only elevated α2-antiplasmin levels were associated with risk, whereas increased TAFI levels even strongly protected against myocardial infarction.[11,40] An explanation for our findings is that the clot lysis time measures the combination of the individual factors, also taking the interplay between them into account. The complex interplay between proteins in the fibrinolytic process is not taken into account when plasma levels of the individual factors are measured. Indeed, we recently showed that the clot lysis time is sensitive for fibrinolytic factors but that the outcome of the assay appears to be determined by factors beyond plasma levels of fibrinolytic proteins.[40]

A limitation of the case-control study design is that levels of the fibrinolytic proteins are measured after the event, and that levels after the event may not reflect levels before the myocardial infarction. Blood samples were drawn several months or even years after the event by which we attempted to minimize the likelihood that an acute-phase reaction was responsible for the plasma levels of the fibrinolytic proteins. Furthermore, we made adjustments for CRP levels, which is an acute-phase protein. Caution is, however, needed in interpreting the results, and replication of our results is required with the use of studies with other study designs, preferably adequately powered prospective studies. From our study we conclude that increased levels of α2-antiplasmin are associated with an increased risk of myocardial infarction in men. PAI-1, t-PA, and plasminogen are no independent risk factors for myocardial infarction. Plasminogen is primarily a marker of inflammation, whereas high PAI-1 and t-PA levels predominantly reflect increased lipid levels and to a lesser extent inflammation. In addition, endothelial activation may in part explain the association between elevated levels of t-PA and myocardial infarction.

Authorship

Contribution: M.E.M. designed the present study, analyzed and interpreted the data, and drafted the manuscript; C.J.M.D. designed the overall study, performed the data collection, interpreted the data, and critically reviewed the analyses and the manuscript; P.G.d.G. interpreted the data and critically reviewed the analyses and the manuscript; F.R.R. designed the overall study, interpreted the data, and critically reviewed the analyses and the manuscript; and T.L. designed the present study, interpreted the data, critically reviewed the analyses, and participated in writing the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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