

# Concentration of Endogenous tPA Antigen in Coronary Artery Disease

## Relation to Thrombotic Events, Aspirin Treatment, Hyperlipidemia, and Multivessel Disease

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**Abstract**—Tissue plasminogen activator (tPA) is the major plasminogen activator responsible for dissolving blood clots found in blood vessels. However, elevated concentrations of tPA antigen were found to be related to adverse events in patients with coronary artery disease (CAD). Considerable controversy about the significance of these results exists. The goal of this cross-sectional study was to identify independent determinants for tPA antigen concentrations in patients with CAD, to possibly clarify the above paradoxical relationship. The baseline tPA antigen concentrations of 366 patients with angiographic evidence of coronary sclerosis were determined. Univariate analysis showed that age ( $P=0.013$ ), angiographic extent of disease ( $P<0.001$ ), presence of angina at rest ( $P<0.001$ ), diabetes mellitus ( $P=0.004$ ), hypercholesterolemia ( $P=0.045$ ), hypertriglyceridemia ( $P=0.015$ ), and chronic intake of nitrates ( $P<0.001$ ) were significantly and positively related to tPA antigen concentration, while the chronic intake of aspirin was inversely related to tPA antigen ( $P<0.001$ ). In addition, plasminogen activator inhibitor type 1 (PAI-1) activity was found to be significantly and positively associated with tPA antigen concentration ( $P<0.001$ ). A multivariate analysis identified chronic low-dose aspirin therapy ( $P<0.001$ ), PAI-1 activity ( $P<0.001$ ), hypertriglyceridemia ( $P=0.005$ ), the type of angina ( $P=0.026$ ), multivessel disease ( $P=0.041$ ), and hypercholesterolemia ( $P=0.043$ ) as significant and independent determinants of tPA antigen. While hypertriglyceridemia and hypercholesterolemia both are related to the underlying disease, the type of angina and the number of involved vessels are linked to the severity and extent of disease, and all of them are indicators of a prothrombotic state found during the progression of CAD. In contrary, low-dose aspirin rather would decrease the likelihood of thrombotic events. The relation of tPA antigen to PAI-1 activity furthermore underlines the relation between tPA antigen concentration and a prothrombotic state. Therefore, the positive or—in case of aspirin therapy—negative correlation of these parameters with tPA antigen concentration would indicate that thrombus formation and simultaneous endothelial cell activation might be major determinants for tPA antigen concentration in CAD. (*Arterioscler Thromb Vasc Biol.* 1998;18:1634-1642.)

**Key Words:** coronary artery disease ■ tissue plasminogen activator ■ aspirin ■ hyperlipidemia

Coronary artery disease (CAD) is frequently complicated by acute coronary syndromes such as unstable angina, acute myocardial infarction, or sudden cardiac death. From angioscopic, angiographic, and autopsy data, it is known that plaque erosion and plaque rupture with consecutive intracoronary thrombus formation up to occlusive thrombosis are key events for the onset of these syndromes.<sup>1-8</sup> However, plaque fissuring is not always followed by occlusive thrombosis. In fact, the frequency of ruptured plaques without occlusive thrombosis detected at autopsy in the coronary arteries of patients who died from CAD<sup>3,4,9,10</sup> indicates that asymptomatic plaque fissuring with only mural thrombosis is a common event in patients with CAD. Such events of silent thrombosis have been implicated in the progression of atherosclerotic

lesions.<sup>11-13</sup> Indeed, according to the concept of “response to injury,”<sup>14</sup> progression of atherosclerosis is due to the action of mitogens and growth factors on the cells within the atherosclerotic plaques. Besides activated platelets, which have long been recognized as a major source of mitogens,<sup>15</sup> active thrombin, as well as fibrin and fibrinogen degradation products, which are generated after activation of the coagulation cascade in response to plaque fissuring, have potent mitogenic properties.<sup>16-18</sup> The extent of thrombosis after rupture of atherosclerotic plaques has been related to local thrombogenic factors.<sup>19</sup> Indeed, an increased local production of prothrombotic<sup>20</sup> and antifibrinolytic<sup>21,22</sup> substances has been demonstrated in atherosclerotic plaques and has also been implicated in progression of atherosclerosis.<sup>23</sup> Together with

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these local alterations of hemostasis and fibrinolysis in the proximity of the plaque, patients with CAD exhibit frequently also a systemic prothrombotic and/or antifibrinolytic state,<sup>24-30</sup> which might contribute to an unfavorable clinical course in case of plaque rupture.<sup>7,23</sup> Accordingly, an insufficient endogenous fibrinolytic system, notably an imbalance between plasma levels of tissue plasminogen activator (tPA) and its main inhibitor plasminogen activator inhibitor type 1 (PAI-1), has been shown to be associated with acute coronary syndromes.<sup>30-32</sup> However, alterations in the fibrinolytic system have also been demonstrated at early stages of atherosclerosis.<sup>33</sup> The importance of the endogenous fibrinolytic system for coronary atherosclerosis is reflected by several recently published retrospective and prospective studies showing that an increased tPA-mass (ie, total tPA-antigen) concentration in plasma is a strong predictor for adverse events in patients with CAD<sup>34-36</sup> as well as for the development of angina in apparently healthy individuals.<sup>37</sup> It is, however, unclear why increased levels of a protein with in principle beneficial properties should be linked to the progression of CAD and a bad prognosis. Increase in tPA might thereby be part of a feedback loop in which thrombin formed at the site of the atherosclerotic plaque would not only lead to local thrombus formation but also activate endothelial cells to release more tPA.<sup>38</sup> On the other hand, tPA circulates in plasma not only free and active, but mainly in complex with PAI-1, and a strong positive correlation between PAI-1 and tPA plasma levels has been reported.<sup>27,33,36,39</sup> Thereby, increased tPA plasma levels could possibly also be a consequence of primarily increased PAI-1 plasma levels. Furthermore, tPA might also be directly responsible for the progression of CAD, as studies from plasminogen knockout experiments in mice indicate that local plasmin formation is a powerful amplifier of arteriosclerotic disease in transplant arteriosclerosis.<sup>40</sup>

The present cross-sectional analysis of tPA antigen concentrations (ie, the sum of free active tPA antigen and tPA bound to PAI-1 in an inactive complex) in 366 consecutive patients with angiographically documented CAD was performed to evaluate the relative importance of PAI-1 activity, coexisting risk factors for coronary atherosclerosis, and other clinical variables as determinants for tPA antigen concentrations in a representative and well-defined study population. In addition, we sought to investigate effects, if any, of usual antithrombotic and antianginous pharmacological therapy (aspirin, nitrates, calcium channel blockers) on tPA levels in relation to clinical variables and accompanying risk factors for coronary atherosclerosis. These data should enable us to identify independent determinants for tPA antigen in patients with CAD to clarify the above paradox relationship between tPA antigen concentrations and prognosis.

## Patients and Methods

### Patients

Three hundred sixty-six consecutive patients of our cardiology department who exhibited coronary sclerosis of different extent as proven by coronary angiography were enrolled into the study. All patients gave written informed consent to take part in the study

**TABLE 1. Baseline Characteristics of the Patients (N=366)**

Clinical Variable	%
Sex (M/F)	79.8/20.2
Age	
<40 years	11.2
41-60 years	53.0
>60 years	35.8
History of previous myocardial infarction	49.6
Type of angina	
Exercise-induced	65.8
Rest	34.2
Coronary angiography	
0-vessel disease	15.5
1-vessel disease	44.7
2-vessel disease	20.5
3-vessel disease	19.3
Risk factors for coronary atherosclerosis	
Smoking	34.1
Arterial hypertension	29.2
Obesity	52.3
Diabetes mellitus	11.6
Hypertriglyceridemia	32.4
Hypercholesterolemia	67.1
Pharmacological treatment	
Nitrates (ISDN)	48.1
Calcium antagonists (nifedipine)	45.9
Aspirin	53.6

Patients were classified according to sex, age ( $\leq 40$  years, 41-60 years, and  $> 60$  years old), history of previous myocardial infarction, type of angina at time of blood sampling (angina induced by physical exercise only or angina at rest), and according to the number of great coronary arteries (left anterior descending, left circumflex, or right coronary artery or major branches thereof) with significant atherosclerotic lesions (ie,  $> 70\%$  narrowing of the original vessel diameter) at time of angiography (0-, 1-, 2-, and 3-vessel disease). Additional classification criteria were the absence or presence of the following risk factors for coronary artery atherosclerosis: chronic cigarette smoking, history of arterial hypertension, obesity, or diabetes mellitus. The plasma levels of total cholesterol and triglyceride were transformed into categorical data using 5.17 mmol/L (200 mg/dL) as upper normal value for total cholesterol and 2.26 mmol/L (200 mg/dL) as upper normal value for triglyceride. As a consequence, patients were classified according to the presence or absence of hypercholesterolemia and hypertriglyceridemia (ie, plasma level above or below 5.17 mmol/L (200 mg/dL) for cholesterol and above or below 2.26 mmol/L (200 mg/dL) for triglycerides, respectively).

according to the Declaration of Helsinki. The detailed characteristics of these patients are given in Table 1.

### Antithrombotic and Antianginous Treatment

As outlined in Table 1, patients were classified according to the presence or absence of an antithrombotic (aspirin) and antianginous (nitrates or calcium channel blockers) therapy for at least 6 weeks' continuous duration before blood sampling. Usually, treated patients received perorally aspirin (100 mg/d), isosorbide dinitrate (ISDN, 2 $\times$ 20 to 40 mg/d in a retard-release form) and sublingual ISDN on request and nifedipine (3 to 4 $\times$ 10 mg/d), respectively.  $\beta$ -Blockers and ACE inhibitors were used only in a minority of these patients and therefore not included into the calculations.

### Blood Sampling

For determination of baseline tPA antigen concentration, blood was drawn after minimal venous stasis from an antecubital vein. In patients with angina at rest, blood sampling was performed immediately at admission and before initiation of any intravenous therapy and/or angiography. Patients with angina at rest were eligible for the study only if blood sampling was performed between 8 and 11 AM. In stable patients who were admitted to the hospital for a routine angiography, blood was drawn before the angiographic procedure within the first 24 to 48 hours after admission between 8 and 11 AM to avoid possible influences of circadian variations on the results.<sup>32,39</sup> Blood sampling was always undertaken before the angiographic procedure to avoid possible influences of catheter-related acute-phase reactions on the assessed fibrinolytic parameters and before any changes of the out-hospital therapy were done. Blood was anticoagulated with EDTA ( $5 \times 10^{-2}$  mol/L final concentration) and plasma was prepared by immediate centrifugation at 3000 rpm for 10 minutes at 4°C. Plasma samples were aliquotted and stored at -70°C until use.

### Determination of tPA Antigen and of PAI-1 Activity Concentrations

tPA antigen concentrations in plasma were determined by means of an ELISA system measuring free tPA as well as tPA/PAI-1 complexes<sup>41,42</sup> (Technoclone Inc). To analyze the relation of tPA antigen concentration to PAI-1 activity, PAI-1 activity in plasma was determined in parallel to the tPA antigen concentration. Determination of PAI-1 activity was performed using an ELISA system measuring only free uncomplexed and active PAI-1<sup>42</sup> (Technoclone Inc). All determinations were done in duplicate, and mean values of the 2 determinations were used for further calculations.

### Determination of Serum Cholesterol and Triglyceride

Total cholesterol and triglyceride levels were determined in heparinized plasma samples drawn in parallel to the tPA samples by use of a Hitachi 747 (Boehringer Mannheim) and commercially available test kits.

### Statistical Analysis

All tPA antigen concentration values were  $\log_{10}$  transformed to resemble a normal distribution. Consequently, all statistical analyses were carried out for  $\log_{10}$ -transformed tPA antigen concentration values. Geometric means and upper as well as lower borders of the 95% confidence interval presented in the tables (unless otherwise indicated) were calculated by exponentiation of the mean values and borders of the 95% confidence interval obtained from the  $\log_{10}$ -transformed tPA antigen concentrations. Differences in tPA antigen concentrations for the various classification variables were investigated by means of the unpaired *t* test or by 1-way ANOVA where appropriate. Multivariate analysis to determine the factors significantly related to the tPA antigen concentration was performed using the ANOVA procedure supplied with the SPSS PC program, version 7.0 (SPSS Inc), including the following variables: sex, age, type of angina, angiographic extent of CAD (this variable was dichotomized using the presence of multivessel disease, ie, 2- or 3-vessel disease, as classification criteria), diabetes, hypertriglyceridemia, hypercholesterolemia, PAI-1 activity (in relation to the median value of the study population), nitrate therapy, and aspirin therapy. A value of  $P < 0.05$  was considered to be statistically significant.

## Results

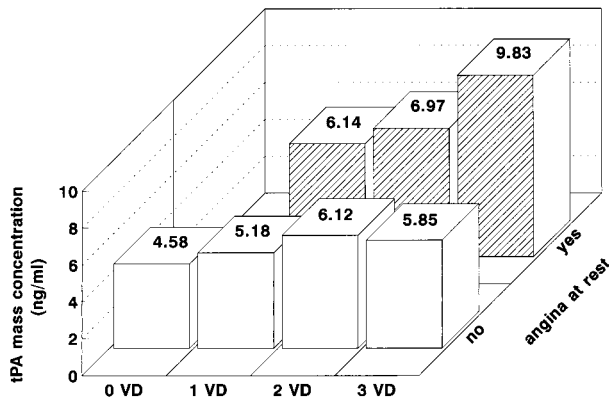
### Baseline Characteristics of the Study Population

Table 1 summarizes the baseline characteristics of the 366 patients according to the classification criteria indicated in the legend. The majority of the patients were between 41 and 60 years old (mean age  $54.8 \pm 11.2$  years) and had a stable form of CAD (ie, exercise-induced angina only) at time of blood

**TABLE 2. Geometric Mean Concentrations of tPA Antigen in Relation to Clinical Variables, Risk Factors for Coronary Artery Atherosclerosis, PAI-1 Activity Levels and Different Pharmacological Treatments**

Clinical Variable	tPA Antigen	95% Confidence Interval		P
		Lower	Upper	
Sex				
M	6.05	5.67	6.46	0.054
F	5.19	4.36	6.18	
Age				
<40	5.14	4.08	6.46	0.013
40-60	5.55	5.10	6.05	
>60	6.63	6.01	7.31	
History of previous MI				
Y	6.22	5.65	6.85	0.083
N	5.56	5.13	6.04	
Angina at rest				
Y	7.48	6.71	8.34	<0.001
N	5.19	4.82	5.58	
Angiographic extent of CAD				
0 VD	4.58	3.85	5.44	<0.001
1 VD	5.56	5.05	6.11	
2 VD	6.32	5.49	7.26	
3 VD	8.11	6.92	9.50	
Smoking				
Y	5.84	5.18	6.58	0.921
N	5.88	5.46	6.33	
Arterial hypertension				
Y	6.38	5.65	7.21	0.096
N	5.68	5.27	6.11	
Obesity				
Y	5.90	5.43	6.40	0.892
N	5.85	5.30	6.45	
Diabetes mellitus				
Y	7.55	6.44	8.86	0.004
N	5.68	5.31	6.08	
Hypertriglyceridemia				
Y	6.84	6.03	7.76	0.015
N	5.55	5.01	6.14	
Hypercholesterolemia				
Y	6.29	5.73	6.92	0.043
N	5.28	4.54	6.14	
PAI-1 activity below median value (7.65 ng/ml)				
Y	4.71	4.35	5.11	<0.001
N	7.32	6.71	7.99	
Therapy with ISDN				
Y	6.60	5.99	7.28	<0.001
N	5.26	4.87	5.69	
Therapy with calcium antagonists				
Y	6.16	5.59	6.79	0.165
N	5.63	5.19	6.11	
Therapy with aspirin				
Y	4.88	4.51	5.28	<0.001
N	7.26	6.63	7.96	

tPA antigen concentration values shown are geometric means with lower and upper border of the 95% confidence interval equally presented. Probability values (2-tailed) were obtained by comparing  $\log_{10}$ -transformed tPA antigen concentrations of the different subgroups using unpaired *t* test or 1-way ANOVA where appropriate.



**Figure 1.** Mean tPA antigen concentrations (geometric means) of patients with CAD in relation to the angiographic extent of disease (0-, 1-, 2- or 3-vessel disease) according to the presence or absence (bars in front) of angina at rest. As there were no patients with angina at rest presenting with 0-vessel disease at time of angiography, only the mean tPA antigen concentrations of patients with 0-vessel disease and angina on exertion can be shown. The number on top of each bar is the mean tPA antigen concentration of the respective group.

sampling. One-third (34.2%) of the patients experienced angina at rest. At the time of angiography, most of the patients exhibited 1-vessel disease (44.7%), whereas multivessel disease (2- or 3-vessel disease) was present in 39.8%. Coronary sclerosis without significant narrowing of major coronary arteries (0-vessel disease) was detected in 15.5% of the patients. Concerning pharmacological therapy, treated and not-treated groups were of comparable size for all 3 therapeutic agents.

### Relation of tPA Antigen Concentration to Clinical Variables and Risk Factors for Coronary Artery Atherosclerosis

Univariate relations between tPA antigen concentrations and different clinical classification criteria as well as risk factors for coronary artery atherosclerosis are shown in Table 2. tPA antigen concentrations increased significantly with increasing age and with an increasing number of significantly stenosed major coronary arteries (see Table 2 for details). In addition, a 1.44-fold higher mean tPA antigen concentration ( $P<0.001$ ) was found for patients exhibiting angina at rest at time of blood sampling than patients experiencing angina only after physical exercise. As shown in Figure 1, the increase in tPA antigen concentration with an increasing number of major coronary arteries involved was found to be independent of the type of angina. Concerning risk factors for coronary artery atherosclerosis, only the presence of diabetes mellitus, hypertriglyceridemia, and hypercholesterolemia were significantly associated with higher tPA antigen concentrations compared with their respective controls ( $P=0.004$ ,  $P=0.014$ , and  $P=0.043$ , respectively).

### tPA Antigen Concentration and Antithrombotic or Antianginous Therapy

In addition, Table 2 demonstrates that patients on an oral therapy with nitrates (ISDN) for at least 6 weeks' duration before blood sampling had significantly higher tPA antigen

**TABLE 3. Significant Determinants of tPA Antigen Following Multivariate Analysis**

Variable	<i>P</i>
Therapy with aspirin	<0.001
PAI-1 activity	<0.001
Hypertriglyceridemia	0.005
Type of angina	0.026
Multivessel disease	0.041
Hypercholesterolemia	0.043

Data depicted are the results from multivariate analysis using ANOVA according to the procedures indicated in the "Methods" section. Only the finally significant variables ( $P<0.05$ ) are listed. No significant interactions between the 6 variables were found. The results of the ANOVA procedure were cross-checked by use of the general linear model (GLM) method for variations in sample size with the SAS program, version 6.03, and were found to be identical.

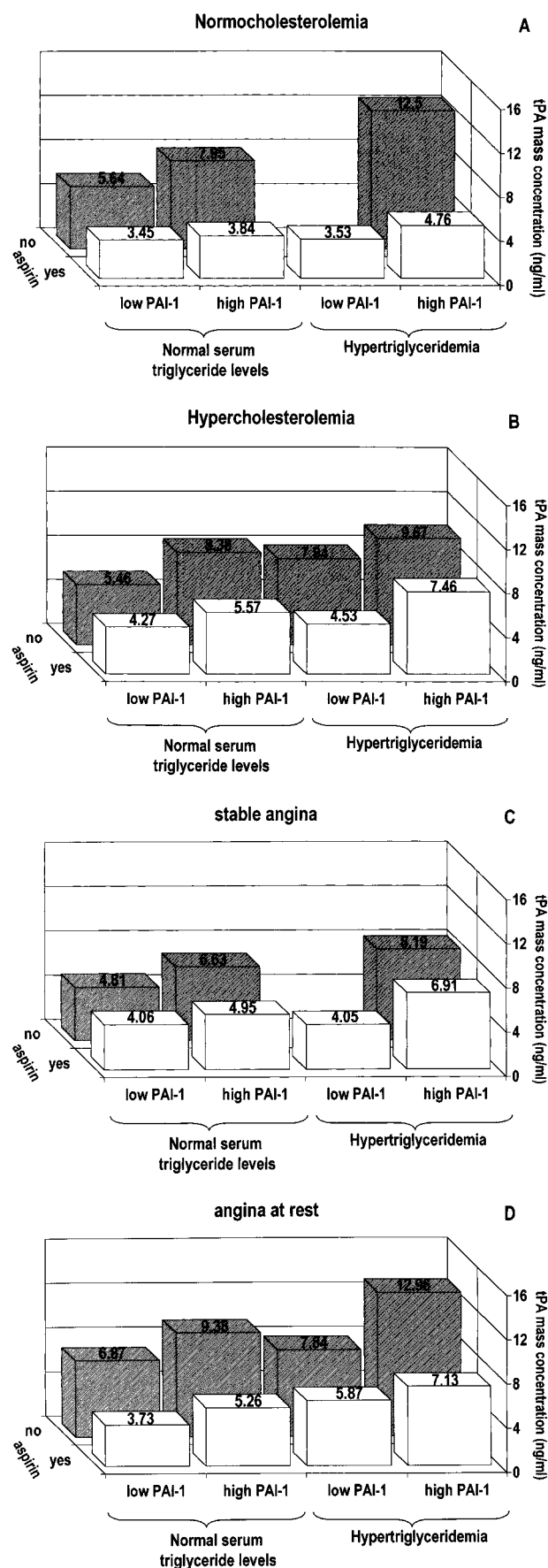
concentrations than patients not taking nitrates. In contrast, patients on low-dose aspirin therapy (100 mg/d) for at least 6 weeks' duration before blood sampling had 1.49-fold lower mean tPA antigen concentrations than patients not taking aspirin ( $P<0.001$ ). No significant differences in tPA antigen concentrations according to calcium antagonist therapy with nifedipine ( $P=0.165$ ) were found.

### tPA Antigen Concentration and PAI-1 Activity

To analyze the dependence of tPA antigen concentration on PAI-1 activity in plasma, plasma levels of PAI-1 activity were determined in parallel to the tPA antigen concentrations, and patients were classified using the median PAI-1 activity value of the study population (7.65 ng/mL) as cutoff point. As seen in Table 2, patients with a PAI-1 activity value below the median value had significantly ( $P<0.001$ ) lower tPA antigen concentrations than patients with a PAI-1 activity above the median value. Accordingly, there was a strong correlation between plasma PAI-1 activity and tPA antigen concentration ( $r^2=0.46$ ,  $P<0.001$ ; data not shown).

### Multivariate Analysis

To identify the significant determinants for tPA antigen concentration in patients with CAD in this study, a multivariate analysis was performed, according to the procedures given in the "Methods" section. Six significant and independent determinants of tPA antigen concentration in our study population were found. From the probability values summarized in Table 3, it can be seen that tPA antigen concentration in patients with CAD was primarily influenced by aspirin therapy ( $P<0.001$ ) and PAI-1 activity ( $P<0.001$ ) followed by the effect of hypertriglyceridemia ( $P=0.005$ ), the type of angina ( $P=0.026$ ), the presence of multivessel disease ( $P=0.041$ ), and finally the presence of hypercholesterolemia ( $P=0.043$ ). No significant 2-way or higher-order interactions between these 6 factors were found. Overall, the model including the above 6 variables explained more than 32% of the variations in tPA antigen concentrations ( $P<0.001$ ).



### Influence of Aspirin Therapy on tPA Antigen Concentration in Relation to the Other Independent Factors

Figure 2 illustrates the reduction of tPA antigen concentrations in patients with CAD by aspirin treatment. Both in subjects with normocholesterolemia (A) and subjects with hypercholesterolemia (B), aspirin treatment is associated with lower mean tPA antigen concentrations than in the respective non-aspirin-treated control groups, independent of the presence of hypertriglyceridemia and the PAI-1 activity level. In addition, in both aspirin-treated and non-treated groups, subjects with angina at rest always had higher tPA antigen concentrations than patients with stable angina (ie, angina on exertion only), as illustrated by Figure 2C (patients with stable angina) and 2D (patients with angina at rest), again independent of the presence of hypertriglyceridemia and of the PAI-1 activity level.

### Discussion

The present cross-sectional study demonstrates that the tPA antigen concentration in patients with CAD is correlated with a chronic treatment with low-dose aspirin (100 mg/d), the PAI-1 activity in plasma, the fasting triglyceride and total cholesterol concentrations, the type of angina at time of blood sampling, and the presence of multivessel disease. These results suggest that the tPA antigen concentration in patients with CAD might be related to thrombotic events in the coronary vessels.

### Evidence for Coronary Thrombosis as a Possible Cause for Elevated tPA Antigen Concentrations in CAD

Several authors have reported increased tPA levels in CAD patients compared with normal control subjects.<sup>27,30,31</sup> In the present study, we found that in patients with angiographic evidence of CAD, the mean tPA levels of patients with angina at rest were significantly (1.44 fold,  $P < 0.001$  by univariate analysis) higher than those of patients with "stable" angina (ie, angina induced only on exercise), as also found by Hoffmeister et al in a recently published study.<sup>30</sup> Our study, however, extends the findings of previous reports by demonstrating that the strong association of angina at rest with tPA antigen concentration persisted after controlling for all other variables ( $P = 0.026$  for angina at rest in multivariate analysis). Acute coronary syndromes, such as unstable angina or myocardial infarction accompanied by angina at rest, are

**Figure 2.** Mean tPA antigen concentrations of patients with normocholesterolemia (total cholesterol plasma level below 5.17 mmol/L [200 mg/dL], A) and hypercholesterolemia (total cholesterol plasma level above 5.17 mmol/L [200 mg/dL], B) according to the presence of hypertriglyceridemia (triglyceride plasma level  $\geq 2.26$  mmol/L [200 mg/dL]), the plasma PAI-1 activity levels (below or above the median value of 7.65 ng/mL), and aspirin therapy. C and D, Mean tPA antigen concentrations of patients with stable angina (angina only on exercise, C) or angina at rest (D) in relation to plasma PAI-1 activity levels (below or above median value of 7.65 ng/mL), presence or absence of hypertriglyceridemia, and presence or absence of aspirin therapy. The number on top of each bar is the mean tPA antigen concentration of the respective group.

frequently associated with occlusive thrombotic events initiated by plaque rupture.<sup>1-8,11,12</sup> Due to this causal relationship between angina at rest and thrombosis, the present data suggest that intracoronary thrombus formation is a strong and independent determinant for tPA antigen concentration in patients with angiographic evidence of CAD. In fact, based on in vitro data demonstrating a reactive release of tPA from the vascular wall after increase in thrombin concentration,<sup>38</sup> activation of thrombin in patients with acute coronary syndromes<sup>43,44</sup> is a possible explanation for systemically elevated tPA antigen concentrations in this subgroup. However, increased thrombin concentrations might explain not only acutely elevated tPA antigen concentrations in patients with angina at rest but also chronically elevated tPA levels in patients with stable CAD compared with healthy control subjects,<sup>27,30,31</sup> since clinically undetected ("silent") thrombotic events have been shown to occur frequently in atherosclerotic arteries.<sup>3,4,9,10</sup> tPA antigen concentration might thereby be considered as a marker of disease activity in patients with CAD.

### **Influence of Antithrombotic Therapy on tPA Antigen Concentration**

A major finding of the present study is that patients taking low-dose aspirin on a chronic basis exhibited significantly lower tPA antigen concentrations than patients without this antithrombotic therapy. This held true in patients with angina at rest as well as in patients with exercise-induced angina and was independent of the presence or absence of hypertriglyceridemia, hypercholesterolemia, the presence of multivessel disease, and PAI-1 activity (Table 3 and Figure 2A through 2D). Accordingly, aspirin therapy was one of the main determinants of tPA-mass ( $P < 0.001$ ) in the multivariate analysis (Table 3). To the best of our knowledge, the present study is the first report demonstrating a potential modulation of tPA antigen concentrations in patients with CAD by aspirin therapy. Our data are supported by results of Ridker et al<sup>37</sup> showing that the predictive value of tPA antigen concentrations for development of angina in apparently healthy men was partially lost when the results were corrected for aspirin treatment and by results of Winther et al<sup>45</sup> demonstrating that aspirin can lower tPA antigen concentrations in healthy individuals, although 3 times higher aspirin doses (300 mg/d) than given in the present study were necessary to obtain this effect. Our results on chronic aspirin therapy and tPA antigen concentration in patients with CAD should be viewed in the context of previously published studies demonstrating an inverse relation between tPA antigen and prognosis in CAD<sup>34-36</sup> and some large multicenter trials that have shown that aspirin therapy is able to reduce the incidence of reinfarction and/or mortality in patients with suspected acute myocardial infarction,<sup>46</sup> the incidence of myocardial infarction in apparently healthy men,<sup>47,48</sup> and the incidence of vascular events in high- and low-risk groups.<sup>49</sup> The favorable effects of aspirin on the prognosis in patients with CAD have been primarily attributed to the antithrombotic/antiplatelet actions of aspirin. Accordingly, the lower mean tPA antigen concentration in the aspirin-treated group found in the present study might be explained by a reduced number of thrombotic

events in the atherosclerotic vessels with decreased reactive tPA release from the vessel wall in response to thrombin formation. However, as aspirin is also an anti-inflammatory drug and inflammation may play a major role in the etiology of atherosclerosis (reviewed in Reference 50), part of the effects of aspirin on tPA antigen concentration might also be linked to this effect. Another alternative explanation for the link between aspirin therapy and lower tPA antigen concentration, namely that patients with less severe CAD might have received aspirin, while patients with more severe CAD might have received other treatments like ISDN, implicating that the severity of disease was the true determinant of tPA antigen concentration, could definitely be excluded based on the multivariate analysis performed, because both aspirin therapy and the type of angina were found to independently determine tPA antigen concentration.

### **Influence of Other Antianginous Treatments on tPA Antigen Concentration**

Therapy with ISDN of more than 6 weeks' duration was found to be a significant parameter for tPA antigen only in univariate analysis and lost its significance after correction for other lipid- and non-lipid-dependent variables. Therefore, the elevation of tPA antigen concentrations in patients treated with ISDN was only an incidental finding probably due to the fact that ISDN had been prescribed more often in symptomatic patients in whom tPA antigen concentrations were already elevated due to recurrent atherothrombosis. A direct and independent effect of ISDN, eg, by its antiaggregatory properties<sup>51</sup> on tPA antigen concentrations, could not be verified in this study. The fact that the calcium antagonist nifedipine had no detectable effect on tPA antigen concentration in the present study might be explained by the lack of antithrombotic and antiplatelet effects of nifedipine in the currently recommended dosage.

### **Relation of Blood Lipids to tPA Antigen Concentrations**

The relation between elevated serum cholesterol levels (reviewed in Reference 52), serum triglyceride levels (reviewed in Reference 53), and atherosclerosis is well established. Like other studies before,<sup>27,29,54</sup> we found that the tPA antigen concentration is strongly dependent on fasting triglyceride levels ( $P = 0.005$ ) and on total cholesterol levels ( $P = 0.043$ ). Several studies have shown a strong correlation of increased plasma levels of PAI-1 with total cholesterol or triglyceride plasma levels<sup>26,27,29,54</sup> and with CAD.<sup>26-31</sup> The increase of PAI-1 in subjects with hypertriglyceridemia has been related to the presence of insulin resistance associated with hypertriglyceridemia.<sup>55</sup> Hypercholesterolemia, in contrast, has been found to increase PAI-1 production in vitro directly.<sup>56</sup> In fact, an enhanced PAI-1 gene expression has been demonstrated in atherosclerotic plaques.<sup>21,22</sup> PAI-1 plasma levels in turn were shown to be significantly correlated with tPA plasma levels in previous studies<sup>27,33,36,39</sup> and in the present analysis. Thereby, increased levels of blood lipids might cause increased tPA antigen concentrations by increasing PAI-1. Based on the multivariate analysis performed, we can exclude this possibility. Indeed PAI-1 activity, the presence of hypertriglycer-

idemia, and the presence of hypercholesterolemia were all three found to independently determine tPA antigen concentration. Therefore hypertriglyceridemia and hypercholesterolemia are likely to influence tPA antigen concentration by different ways than by increasing PAI-1 activity. One possible explanation is the increased thrombotic tendency found in association with hypertriglyceridemia and hypercholesterolemia,<sup>57-59</sup> with the higher rate of local thrombus formation causing elevations in tPA antigen concentration. In addition, lipids might also exert a direct effect on tPA expression, but such direct effects have not been proven up to now.

### Relation of PAI-1 and tPA Antigen Concentration

The tPA antigen concentration measured in this and other studies<sup>27,34-37</sup> is the sum of free active tPA and of tPA bound to inhibitors like PAI-1 in an inactive complex. Therefore, an increase in tPA antigen concentration in patients with CAD might be due to an increase in PAI-1, with secondary formation of more stable tPA/PAI-1 complexes, as already suggested by Jansson et al<sup>35</sup> and Thompson et al.<sup>36</sup> In effect, the strong positive correlation of tPA antigen concentration and PAI-1 plasma levels in this and other studies<sup>27,33,36,39</sup> might support this conclusion. Alternatively, the tPA antigen concentration might be reactively elevated as part of an autoregulatory feedback loop in response to elevated PAI-1 plasma levels, whereby PAI-1 induces an increase in tPA release from endothelial cells either directly or via increased local thrombin formation.<sup>38</sup>

### tPA Antigen Concentration and Angiographic Extent of Coronary Artery Disease

Similar to the results reported by the ECAT angina pectoris study group,<sup>29,36</sup> we found in the present study a significant and positive association between the number of more than 70%-stenosed coronary arteries and tPA antigen concentration. Figure 1 demonstrates that the increase of tPA antigen concentration with an increasing number of significantly stenosed coronary arteries is independent of the disease activity, because such an increase of tPA antigen concentration is seen in patients with and without angina at rest. When patients were classified according to the presence or absence of multivessel disease, the angiographic extent of CAD was even found to be a significant ( $P=0.041$ ) determinant of tPA antigen concentration, independent of hypertriglyceridemia, hypercholesterolemia, aspirin therapy, PAI-1 activity, and angina at rest (Table 3). Therefore, tPA antigen concentration can serve not only as a marker of disease activity but also as a marker of disease extent.

### Limitations of the Study and Alternative Explanations for Increased tPA Antigen Concentrations in Patients With CAD

Hyperinsulinemia has been reported to be an independent risk factor for ischemic heart disease.<sup>60</sup> The fact that patients with diabetes have represented only 11.6% in the study population might, however, be responsible for the fact that diabetes could not be identified as independent factor for tPA antigen concentrations in CAD in the present study. Moreover, as the 6 significant variables for tPA antigen concentration in

patients with CAD identified in the present study explained only 32.7% of the total variations in tPA antigen concentrations ( $P<0.001$ ), other factors not analyzed by the present study (eg, homocysteine, Lp(a), HDL cholesterol) and potentially not yet identified might be strong predictors of tPA antigen concentrations as well. Furthermore, while we have pointed to the strong relation between tPA antigen concentrations and coronary thrombotic events in our study, it cannot be excluded that the independent determinants of tPA antigen concentrations identified in the present study might influence tPA antigen concentrations via different mechanisms. In effect, aspirin is only a weak platelet inhibitor,<sup>61</sup> and while the effects of stronger antiplatelet drugs on tPA antigen concentrations have to be evaluated to confirm this study, an alternative explanation for the observed effects of aspirin therapy on tPA antigen concentration in patients with CAD might be found in the anti-inflammatory effects of aspirin. Indeed progression of coronary artery disease has been linked to inflammatory events (reviewed in Reference 50). Plaque rupture per example was found to be related to macrophage infiltration of plaques (reviewed in Reference 7). Cell-bound plasminogen activators and plasminogen activators upregulated by macrophages<sup>62</sup> might activate matrix-degrading enzymes by virtue of plasmin activation, thereby weakening the plaque, predisposing to plaque rupture<sup>7</sup> and enhancing disease progression. In addition, immunohistochemical studies have found increased tPA within atherosclerotic lesions<sup>63,64</sup> and excess extractable tPA activity from atherosclerotic coronary arteries.<sup>65</sup> While tPA might act as an autocrine growth factor on human aortic smooth muscle cells<sup>66</sup> and therefore predispose to plaque growth, the increased plasminogen activator activity within an atherosclerotic plaque might also result in an increased degradation of fibrin.<sup>16</sup> Fibrin degradation products, however, have strong mitogenic properties,<sup>18</sup> and it should be remembered in this context that fibrin degradation products have been found to be associated with an increased risk of future myocardial infarction.<sup>67</sup>

### Conclusion

In this study, 6 independent variables have been identified that significantly determine tPA antigen concentrations in patients with documented CAD. In view of recent data that report an association between elevated tPA antigen concentrations and poor prognosis in patients with CAD, our study provides data supporting the view that the prognostic importance of tPA in CAD reflects disease activity and might be linked to recurrent thrombotic events. However, alternative explanations of increased tPA antigen concentrations in CAD (eg, inflammatory processes within the atherosclerotic plaques, endothelial damage) cannot be excluded. In addition, our study indicates that elevated tPA antigen concentrations in patients with CAD might be a marker for multivessel disease, and determination of tPA antigen concentrations in patients with CAD might be useful to discriminate between patients with multivessel disease and patients with single- or 0-vessel disease.

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