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Short communication

Plasminogen activator inhibitor-1 and tissue plasminogen activator and incident AF: Data from the PREVEND study

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ABSTRACT

Background: Tissue-type plasminogen activator (TPA) and plasminogen activator inhibitor-1 (PAI-1) have been shown to be associated with increased risk for stroke, however, the association with incident atrial fibrillation (AF) is unknown.

Methods: We examined 8265 individuals included in the PREVEND study. In all subjects, PAI-1 and TPA plasma/serum levels were measured using an ELISA kit. By means of univariate and multivariate analyses the association with incident AF was assessed.

Results: Mean age was 49 ± 13 years, and 50% were women. Of the 8265 patients free of AF at baseline, 267 (3.2%) patients developed incident AF during a mean follow-up of 9.7 ± 2.4 years. Baseline median levels of PAI-1 and TPA were 72.4 ng/ml and 3.1 ng/ml, respectively. In univariate analyses PAI-1 (Hazard ratio [HR] 1.10 95% Confidence interval [CI] 1.04–1.16, $p < 0.001$) and TPA (HR 1.05, 95% CI 1.01–1.08, $p = 0.014$) were associated with incident AF. However, after multivariate adjustment for age, sex, antihypertensive drugs, stroke, heart failure, myocardial infarction, diabetes mellitus, peripheral arterial disease, smoking, NT-proBNP, alcohol consumption, body mass index no significant association was found.

Conclusion: In this community-based cohort, PAI-1 and TPA levels were not associated with incident AF.

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1. Introduction

Atrial fibrillation (AF) is a common cardiac arrhythmia and is a major public health problem [1,2]. It has a lifetime risk of one in four for persons over the age of 55 years in the Netherlands [1,2]. AF itself is associated with a substantial risk of stroke or thrombo-embolism [3]. The other way around, recent experimental data suggest that hypercoagulability has the potential to cause or promote AF [4]. Tissue-type plasminogen activator (TPA) is synthesized by endothelial cells and activates the profibrinolytic proenzyme plasminogen. Plasminogen activator inhibitor-1 (PAI-1) inhibits the activity of TPA and thereby the fibrinolytic process. Both are markers of fibrinolysis and are risk factors for thrombosis and atherosclerosis [5,6]. In several patient categories it is shown that an increased level of TPA or PAI-1 is associated with

cardiovascular outcome and mortality. For instance healthy individuals who have high levels of antigen TPA have a higher risk of future stroke [7]. Cross-sectional studies in patients with prevalent AF reported elevated levels of TPA or PAI-1 in these patients [8,9]. Furthermore, in patients with prevalent AF, increased levels of TPA have been associated with an increased risk of major adverse events (e.g. transitory ischemic attack, stroke, myocardial infarction) and all-cause mortality [10].

It is, however, unknown whether increased levels of PAI-1 or TPA in the general population, who did not have AF at baseline, are associated with an increased risk for incident AF. Therefore we investigated in a contemporary cohort of individuals without AF whether increased levels of PAI-1 or TPA are associated with an increased risk for incident AF during follow-up.

2. Methods

2.1. Population

The PREVEND was founded in 1997 and includes a cohort of 8592 individuals [11]. The inclusion and validation of patients who developed AF have been described before [2,11].

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Briefly, 7786 individuals with a urinary albumin excretion > 10 mg/L and 3395 individuals with a urinary albumin excretion < 10 mg/L were invited to the PREVEND outpatient clinic. The final cohort consisted of 8592 individuals. At the baseline visit, in addition to detailed information about demographics, health behaviors, anthropometric measurements, cardiovascular, and metabolic risk factors, also blood samples and two 24-h urine samples on 2 consecutive days were collected. For present analysis, individuals without any standard 12-lead ECG (n = 248) were excluded, and those with prevalent AF (n = 79). For the final analysis 8265 individuals were included. The measuring of PAI-1 and TPA has been described before [12]. In brief, in PREVEND blood was drawn and anticoagulated with EDTA and stored at -80°C . Plasma antigen levels of PAI-1 and t-PA were measured using an ELISA kit from Technoclone GmbH (Vienna, Austria) [12]. The PREVEND study was approved by the institutional medical Ethics Committee and conducted in accordance with the Declaration of Helsinki. All individuals provided written informed consent.

2.2. Follow-up

The follow-up duration was calculated as the time between the baseline visit to the last contact date, death, or 31 December 2008, whichever came first.

2.3. Incident atrial fibrillation and covariates

Incident AF ascertainment and covariate definitions have been previously described [2,13]. Briefly, incident AF was diagnosed if either atrial flutter or AF was present on a 12-lead ECG obtained at one of the three PREVEND follow-up visits or at an outpatient visit or hospital admission in the two hospitals in the city of Groningen.

2.4. Statistical analysis

A statistical weighting method was used in the prespecified Cox proportional-hazards regression analyses, to adjust the overselection of individuals with microalbuminuria at baseline, and allow generalization of results to the general population [13]. Individual characteristics were presented as mean \pm standard deviation or median (range) for continuous variables and counts with percentages for categorical variables. We first performed an univariate analysis with PAI and TPA as continuous variables and subsequently a multivariate analysis which adjusted for age, sex, antihypertensive medication, previous stroke, prevalent heart failure, previous myocardial infarction, diabetes mellitus, peripheral arterial disease, smoking, NT-proBNP, alcohol use >2 units/week, and body mass index (as previously described [2]). The proportionality assumption was checked by calculating the Schoenfeld residuals, and where needed time-varying covariates were included to avoid proportionality violations. All analyses were performed using R package (version 3.03), and a p value < 0.05 was considered as statistically significant.

3. Results

The present population contained 8265 individuals with a mean age of 49 ± 13 years, half of them were women (50.2%). Individual characteristics are shown in Table 1. In total, 267 (3.2%) of 8265 individuals developed incident AF during 9.7 ± 2.4 years of follow-up. Baseline

Table 1
Population characteristics.

Characteristic	Total population (n = 8265)	Incident AF (n = 267)	No AF (n = 7998)	p-Value (incident AF versus no AF)
Age (years) - mean \pm SD	49 \pm 13	62 \pm 9	49 \pm 13	<0.001
Male sex - no. (%)	4120 (49.8)	187 (70.0)	3933 (49.2)	<0.001
Body mass index (kg/m ²) - mean \pm SD	26.1 \pm 4.2	27.9 \pm 4.2	26.0 \pm 4.2	<0.001
Systolic blood pressure (mmHg) - mean \pm SD	129 \pm 20	143 \pm 23	129 \pm 20	<0.001
Diabetes - no. (%)	310 (3.8)	24 (9.3)	286 (3.6)	<0.001
Previous myocardial infarction - no. (%)	251 (3.1)	41 (16.1)	210 (2.7)	<0.001
Prevalent heart failure - no. (%)	18 (0.2)	6 (2.2)	12 (0.2)	<0.001
Previous stroke - no. (%)	81 (1.0)	9 (3.4)	72 (0.9)	0.001
Peripheral artery disease - no. (%)	291 (3.7)	28 (11.2)	263 (3.4)	<0.001
Antihypertensive medication - no. (%)	1098 (16.1)	100 (43.9)	998 (15.2)	<0.001
Oral antidiabetic medication - no. (%)	115 (1.7)	11 (4.8)	104 (1.6)	0.002
Statin - no. (%)	333 (4.9)	32 (14.0)	301 (4.6)	<0.001
Total cholesterol (mmol/L) - median (range)	5.6 (4.9–6.3)	5.8 (5.2–6.6)	5.5 (4.9–6.3)	<0.001
Glucose (mmol/L) - median (range)	4.7 (4.3–5.1)	5.0 (4.6–5.6)	4.7 (4.3–5.1)	<0.001
NT-proBNP (ng/L) - median (range)	37.3 (16.6–72.6)	103.4 (44.4–249.5)	36.0 (16.2–70.0)	<0.001
Creatinin (umol/L) - median (range)	82.0 (74.0–92.0)	88.0 (78.0–98.0)	82.0 (73.0–92.0)	<0.001
eGFR (ml/kg/min) median (range)	80 (71–90)	75 (68–84)	80 (72–90)	<0.001
Cystatin C (mg/L) - median (range)	0.8 (0.8–0.9)	0.9 (0.8–1.0)	0.8 (0.7–0.9)	<0.001
High sensitive CRP (mg/L) - median (range)	1.3 (0.6–2.9)	2.0 (0.8–3.6)	1.3 (0.6–2.9)	<0.001
PAI-1 (ng/ml) - median (range)	72.4 (41.7–124.8)	98.7 (55.8–160.8)	71.9 (41.2–123.3)	<0.001
TPA (ng/ml) - median (range)	3.1 (2.3–4.7)	3.7 (2.6–5.5)	3.1 (2.3–4.6)	<0.001
TPA:PAI-1 ratio (range)	0.048 (0.027–0.081)	0.050 (0.029–0.078)	0.048 (0.027–0.081)	0.635

AF = atrial fibrillation, NT-proBNP = N-terminal Pro-brain natriuretic peptide, PAI-1 = plasminogen activator inhibitor, TPA = tissue plasminogen activator.

Table 2
Univariate and multivariate analyses.

Variable	Hazard ratio (95% CI)	p-Value
<i>Univariate</i>		
PAI-1 (per 50 ng/ml)	1.10 (1.04–1.16)	<0.001
TPA (per 5 ng/ml)	1.05 (1.01–1.08)	0.014
TPA:PAI-1 ratio (per 0.1 units)	0.88 (0.73–1.07)	0.212
<i>Multivariate</i>		
PAI-1 (per 50 ng/ml)	1.03 (0.94–1.13)	0.548
TPA (per 5 ng/ml)	1.03 (0.97–1.10)	0.296
TPA:PAI-1 ratio (per 0.1 units)	0.97 (0.81–1.17)	0.742

PAI-1 = plasminogen activator inhibitor, TPA = tissue plasminogen activator. Multivariate analysis adjusted for: age, sex, antihypertensive drugs, stroke, heart failure, myocardial infarction, diabetes mellitus, peripheral arterial disease, smoking, NT-proBNP, alcohol consumption, body mass index.

median levels of PAI-1 and TPA were 72.4 ng/ml and 3.1 ng/ml, respectively. In univariate analyses, PAI-1 (Hazard ratio [HR] 1.10 95% Confidence interval [CI] 1.04–1.16, $p < 0.001$) and TPA (HR 1.05, 95% CI 1.01–1.08, $p = 0.014$) were associated with incident AF. However, after multivariate adjustment for age, sex, antihypertensive drugs, stroke, heart failure, myocardial infarction, diabetes mellitus, peripheral arterial disease, smoking, NT-proBNP, alcohol use > 2 units/week, and body mass index, no significant association was found (Table 2).

3.1. Sensitivity analysis

In addition to PAI-1 and TPA the TPA:PAI-1 ratio was analyzed which was not associated with incident AF (Table 2). Also additional gender specific analysis generated no significant p-values: interaction p-values for male versus females, for TPA, PAI-1 and TPA:PAI-1 ratio were 0.87, 0.48, and 0.37 respectively. The same holds for age which had no influence on the association of incident AF.

4. Discussion

The present analysis showed that increased levels of PAI-1 and TPA were not associated with an increased risk of incident AF, after multivariable adjustment, in a large contemporary, community-based cohort free of AF at baseline. Earlier reports showed that PAI-1 and TPA were associated with thromboembolic outcomes in patients with known

cardiovascular disease [7]. Only limited data is available concentrating on fibrinolytic function in patients who have AF. The fibrinolytic system and its relation to AF and its associated underlying mechanisms are complex and not completely understood [14]. Earlier reports in patients with lone AF suggested that AF itself modified fibrinolytic markers [15]. Also PAI-1 levels were found predictive of a successful cardioversion, and considered as an independent predictor for AF after coronary artery bypass surgery [16,17].

What did the increased levels of PAI-1 and TPA mean in patients who developed AF in the present population. It may mirror early consequences of endothelial damage or dysfunction or a form of systemic inflammation, which can lead to AF in the years thereafter [18]. Another potential explanation is that it might be due to inflammation, endothelial damage, and fibrinolysis or due to confounders (hypertension or insulin resistance) or a combination [14]. Intriguing as inflammation plays an important role in the development of AF and inflammation may rise from many associated conditions (i.e. obesity, hypertension, coronary artery disease) [19]. Additionally it could be related to structural remodeling of the atria, by means of fibrosis, and even could induce electrical remodeling of the atria, which could eventually lead to AF. Why was PAI-1 and TPA not multivariable associated with future AF in the present study? As the multivariate analyses indicated it is likely due to cardiovascular risk factors and diseases which are associated with the increased levels and could therefore be considered as potential confounder. For example, hypertension, heart failure, or ischemic heart disease can all lead to endothelial damage or inflammation [6,18]. Also the PREVENT population is rather young with a mean age of 49 years. As experimental studies have shown that hypercoagulability promotes the development of a substrate for AF and AF progression [4]. Additional studies on the role of coagulation and fibrosis and their role in atrial remodeling and development of AF are evidently needed [20].

4.1. Strengths and limitations

Strengths of our study are the large and contemporary community-based cohort, with a detailed clinical assessment and a strong validation of incident AF and cardiovascular events. Most limitations are the result of the observational design of the community-based cohort study. Probably not all asymptomatic paroxysmal AF episodes are captured as we did not have continuous ECG recordings. Furthermore, the number of individuals with incident AF was modest, which reduced our statistical power to detect significant associations between PAI-1 or TPA and incident AF.

5. Conclusions

In this large contemporary cohort PAI-1 and TPA levels were not associated with the onset of AF.

Conflict of interest

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