Increased tissue plasminogen activator levels in patients with nonvalvular atrial fibrillation

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Abstract

Objective: To determine whether plasma tissue plasminogen activator (tPA) levels (a) are higher in patients with nonvalvular atrial fibrillation (NVAF) than in control subjects in sinus rhythm; (b) differ between NVAF patients with and without a history of an embolic event (transient ischemic attack or embolic stroke); and (c) differ in control subjects with and without a history of thrombotic stroke.

Design: Cross-sectional study.

Setting: Internal medicine outpatient group practice and anticoagulation clinic in 2 teaching hospitals.

Patients: Seventy-four NVAF patients (24 with and 50 without a history of an embolic event), separated into 3 groups: no prior embolic event and no warfarin use (group 1), no prior embolic event and warfarin use (group 2), and prior embolic event and warfarin use (group 3). Forty control subjects in sinus rhythm (29 without and 11 with prior thrombotic stroke).

Outcome measures: Plasma tPA levels.

Results: The age-adjusted mean tPA levels exceeded the upper limit of normal in all 3 NVAF groups but not in the control groups. The NVAF patients had significantly higher mean tPA levels than the control subjects ($p = 0.015$). The levels did not differ significantly between the NVAF patients with a history of an embolic event and those without such a history. The control subjects with a history of thrombotic stroke had significantly higher mean tPA levels than the other control subjects ($p = 0.03$).

Conclusions: NVAF patients, regardless of their history of embolic events, and control patients with a history of thrombotic stroke have higher tPA levels than subjects in sinus rhythm without a history of stroke. A prospective, longitudinal study involving NVAF patients is required to determine whether high baseline tPA levels are associated with, and perhaps causally related to, an increased risk of stroke.

Résumé

Objectif : Déterminer si les taux plasmatiques d'activateur tissulaire du plasminogène (a) sont plus élevés chez les patients en fibrillation atriale non vasculaire (FANV) que chez des sujets témoins en rythme sinusal; (b) diffèrent entre les patients en FANV qui ont ou n'ont pas déjà été victimes d'une embolie (accident ischémique transitoire ou embolie); et (c) diffèrent chez les sujets témoins qui ont ou n'ont pas déjà subi une thrombose.

Conception : Étude transversale.

Contexte : Pratique collective externe en médecine interne et clinique d'anticoagulation dans 2 hôpitaux d'enseignement.

Patients : Soixante-quatorze patients en FANV (24 qui avaient déjà subi une embolie et 50 qui n'en avaient pas subi), séparés en 3 groupes : aucune embolie antérieure et aucune utilisation de la warfarine (groupe 1), aucune embolie antérieure et utilisation de la warfarine (groupe 2) et embolie antérieure et utilisation de la warfarine (groupe 3). Quarante sujets témoins en rythme sinusal (29 qui n'avaient pas été victimes d'une thrombose auparavant et 11 qui en avaient subi une).

Evidence

Études

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Mesures des résultats : Taux d'activateur tissulaire du plasminogène.

Résultats : Les taux plasmatiques moyens corrigés selon l'âge d'activateur tissulaire du plasminogène ont dépassé la limite supérieure de la normale chez les 3 groupes de sujets en FANV mais non chez les sujets témoins. Les patients en FANV avaient des taux moyens d'activateur tissulaire du plasminogène beaucoup plus élevés que les sujets témoins (p = 0,015). Il n'y avait pas de différence importante entre les taux des patients en FANV qui avaient déjà été victime d'une embolie et ceux des patients qui n'en avaient pas subi. Les sujet témoins qui avaient été victimes d'une thrombose présentaient des taux moyens d'activateur tissulaire du plasminogène beaucoup plus élevés que les autres sujets témoins (p = 0,03).

Conclusions : Les patients en FANV, qu'ils aient ou non été victimes d'une embolie, et les patients témoins qui ont déjà été victimes d'une thrombose présentent des taux d'activateur tissulaire du plasminogène plus élevés que les sujets en rythme sinusal qui n'ont pas subi d'attaque. Il faut procéder à une étude longitudinale prospective de patients en FANV pour déterminer s'il y a un lien, de cause à effet, entre des taux de base élevés d'activateur tissulaire du plaminogène et un risque accru d'attaque.

Nonvalvular atrial fibrillation (NVAF) is associated with an annual risk of stroke of 5%.[1,2] Although clinical factors such as congestive heart failure, hypertension and age are independent predictors of stroke in NVAF patients,[3,4] the cause of stroke is assumed to be an embolism arising from a thrombus in the fibrillating left atrium. Abnormalities of various components of the clotting system have been found in cross-sectional studies involving patients with atrial fibrillation.[5–7]

Recently, elevated levels of endogenous tissue plasminogen activator (tPA), an important mediator of intravascular fibrinolysis, have been shown in longitudinal studies to be predictive of myocardial infarction[8] and stroke.[9] This mediator has not previously been studied specifically in NVAF patients. We thus conducted this study to determine whether tPA levels are elevated in patients with NVAF, in comparison with control subjects in sinus rhythm, and whether they differ among NVAF patients with and without a history of an embolic event (transient ischemic attack or embolic stroke). We also examined tPA levels in a small group of control subjects with a history of thrombotic stroke.

Methods

This study was an offshoot of a study in which we examined abnormalities in clotting factors and products in patients with NVAF and in control subjects in sinus rhythm with and without a history of thrombotic stroke (see page 673). After the first study was completed, data were published that suggested a relation between endogenous tPA levels and stroke.[9] Because we had banked an extra aliquot of frozen plasma from the subjects in our main study, we were able to examine whether the clotting activation in NVAF subjects was matched by activation of clot lysis, as reflected in their tPA levels.

The methods of recruiting the NVAF subjects and the control subjects, and the exclusion criteria used, are described in the first study. Informed consent was obtained from all participants, and the study protocol was approved by the hospitals' ethics committees. The NVAF patients were separated into 3 groups: those with no prior embolic event and no warfarin use (group 1), no prior embolic event and warfarin use (group 2), and a prior embolic event and warfarin use (group 3).

All study participants had blood drawn by venipuncture by the same study nurse. Within 30 minutes of venipuncture, plasma fractions were obtained by centrifugation at 4°C for 30 minutes at 2500 rpm. The plasma aliquots were stored at –70°C until use. The plasma tPA level was measured by enzyme-linked immunoassay with a kit from Biopool Canada (Burlington, Ont.).

Frozen plasma samples were no longer available for 3 of the original study subjects. These subjects were therefore excluded from the current study.

Statistical analysis

For age and tPA levels, means and standard deviations (SDs) are given; for the tPA levels the minimum and maximum values are also provided. The difference in mean age between groups was tested using 2-tailed t-tests. The significance of differences in tPA levels between groups was tested using one-way analysis of variance with adjustment for age. A p value of less than 0.05 was considered significant.

Results

Plasma samples were available for 114 of the original 117 study subjects: 74 patients with chronic NVAF, and 40 control subjects (29 without and 11 with a history of thrombotic stroke). Of the NVAF patients 40 had no history of an embolic event, 29 of whom were taking warfarin, and 24 had a history of an embolic event, all of whom were taking warfarin.

The clinical and demographic data of the patient groups are shown in Table 1. The mean ages of patients in groups 1 and 3 were significantly higher than those of the control subjects without a history of stroke ($p = 0.002$ and $p < 0.001$, respectively). Because the tPA level is known to increase with age,[10] all subsequent analyses were age-adjusted.

Table 2 lists the mean tPA levels for the NVAF patient groups and the control groups. The laboratory reference range for the tPA level is shown at the bottom of the table. The mean level was higher in the 3 NVAF groups combined than in the control group without a history of stroke ($p = 0.015$). The mean levels did not differ significantly between the NVAF patients without a history of an embolic event and those with such a history (i.e., groups 1 and 2 v. group 3; data not shown). Among the control subjects, the group with a history of stroke had a higher mean tPA level than the group without such a history ($p = 0.03$). Fig. 1 shows the median tPA levels and the interquartile ranges for the 5 patient groups.

Discussion

We found that NVAF patients had significantly higher mean tPA levels than control subjects in sinus rhythm, even after adjustment for differences in age distribution between patient groups. We also found that within all 3 subgroups of NVAF patients, the mean tPA levels exceeded the upper limit of the laboratory reference range; the tPA levels were unaffected by warfarin use or a history of an embolic event. Finally, we found higher mean tPA levels in the small group of control subjects with a history of thrombotic stroke than in the control group without a history of stroke.

Previous studies have examined the relation between endogenous tPA levels and stroke. In the Physician's Health Study, healthy physicians who subsequently had first-ever strokes after an average follow-up of 5 years had significantly higher mean tPA levels at baseline than control subjects matched for age and smoking status.[9] This finding persisted even after controlling for stroke risk factors such as hyperlipidemia, diabetes, blood pressure,

Table 1: Mean age of patients with nonvalvular atrial fibrillation (NVAF) and of control subjects in sinus rhythm

Patient group	No. of patients	Mean age (and SD*), yr
NVAF patients		
Group 1: no prior embolic event, not taking warfarin	29	73.0 (9.9)†
Group 2: no prior embolic event, taking warfarin	21	70.3 (9.4)
Group 3: prior embolic event, taking warfarin	24	74.3 (6.9)‡
Control subjects		
No prior thrombotic stroke	29	65.7 (11.5)
Prior thrombotic stroke	11	64.7 (14.0)

*SD = standard deviation.
†$p = 0.002$, compared with control subjects without history of stroke.
‡$p < 0.001$, compared with control subjects without history of stroke.

Table 2: Mean endogenous tissue plasminogen activator (tPA) levels and range

Patient group	tPA level, ng/mL	
	Mean (and SD)	Range*
NVAF patients		
Group 1	13.1 (4.9)†	3.8–23.0
Group 2	15.6 (6.1)†	7.4–35.0
Group 3	13.5 (4.8)†	5.6–25.6
Control subjects		
No prior thrombotic stroke	10.6 (3.5)	3.7–18.4
Prior thrombotic stroke	14.5 (8.2)‡	4.2–30.1

*Laboratory reference range 0.1–11.7 ng/mL.
†$p = 0.015$, compared with control subjects with no prior thrombotic stroke, by analysis of variance (ANOVA) after adjustment for age.
‡$p = 0.03$, compared with control subjects with no prior thrombotic stroke, by ANOVA after adjustment for age.

body mass index and family history of premature myocardial infarction. Most (80.7%) of the 88 cases of stroke were reported to be thromboembolic; however, there were no data provided on the relation between atrial fibrillation and tPA levels. The mean tPA level in the control subjects was 9.59 ng/mL, similar to that in our control subjects; in the patients with stroke it was 11.4 ng/mL, lower than that found in our NVAF patients. This suggests that the high tPA levels found in our NVAF patients could prove to be an important factor in the pathogenesis of stroke. In a cross-sectional study of patients attending a metabolic outpatient ward, endogenous tPA was found to be elevated in patients with a history of ischemic stroke.[11] However, because of the study design it is unclear whether the elevated levels were a consequence or a cause of stroke.

Endogenous tPA has been studied in atherosclerotic heart disease. In a nested case–control study within the Physician's Health Study, the baseline mean tPA levels were significantly higher in healthy participants in whom myocardial infarction subsequently developed than in those in whom it did not.[8] This difference was lost, however, after adjustment for risk factors for atherosclerosis, which suggests that tPA may be a marker of preclinical atherosclerosis. In a 2-year follow-up study involving 3043 patients with angina pectoris, baseline levels of tPA, and of fibrinogen and von Willebrand factor antigen, were found to be independent predictors of subsequent myocardial infarction or sudden cardiac death, after adjustment for degree of coronary artery disease and coronary risk factors.[12] In that study the difference in mean tPA levels between groups was only 10%, less than the differences noted in our study. A smaller, 7-year follow-up study involving patients with angina pectoris and angiographically proven coronary artery disease demonstrated that an increased tPA level at baseline was related to a higher risk of death, even after adjustment for other risk factors.[13]

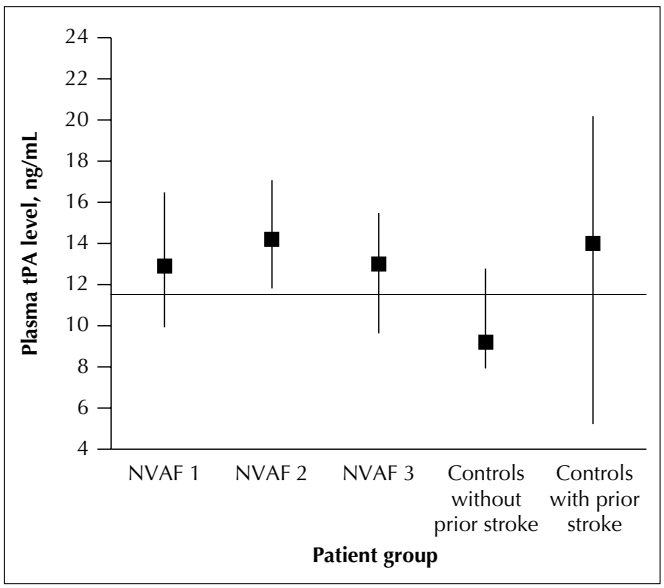

Fig. 1: Median plasma tissue plasminogen activator (tPA) levels (boxes) and interquartile ranges (vertical rules) in patients with nonvalvular atrial fibrillation (NVAF) and control subjects in sinus rhythm with and without a history of thrombotic stroke. NVAF 1 = patients with no prior embolic event (transient ischaemic attack or embolic stroke) and not taking warfarin, NVAF 2 = patients with no prior embolic event and taking warfarin, NVAF 3 = patients with prior embolic event and taking warfarin. Horizontal rule across the middle of the graph denotes laboratory upper limit of normal for tPA levels (11.7 ng/mL).

An elevated tPA level is thus a strong indicator of thrombotic risk. Since endogenous tPA reflects fibrinolytic activity, it seems counterintuitive that elevated levels would be associated with stroke and coronary events. In fact, in the Northwick Park Heart Study, a strong independent relation was found between *low* fibrinolytic activity and a higher risk of coronary events.[14] This is in contrast to the high levels found in our study and other studies. It is known that, in plasma, circulating tPA forms a 1:1 molecular complex with its fast-acting inhibitor, plasminogen-activator inhibitor type 1 (PAI-1). Since both free and bound tPA are measured together, elevated tPA levels could reflect an increase in tPA–PAI-1 circulating complexes, which would be a marker for low intrinsic fibrinolytic activity.[15,16] It remains unresolved as to whether elevated tPA and PAI-1 levels are the result of endothelial dysfunction, represent a net inhibition of fibrinolysis or represent activation of fibrinolysis in response to underlying atherosclerosis or thrombosis.[17]

Our study has certain limitations that could have influenced the validity of the results. The small samples accord limited power to eliminate real differences between subgroups of NVAF patients. Also, the cross-sectional design makes it difficult to determine whether the elevated tPA levels were induced by atrial fibrillation or predated the development of atrial fibrillation.

In summary, we found that tPA levels were higher in NVAF patients than in control subjects without a history of stroke, whose levels were within normal laboratory limits. We also found that they were higher in control subjects with a history of thrombotic stroke than in control subjects without such a history. Our study design was unique in that we examined subgroups of NVAF patients taking and not taking warfarin, with and without a prior embolic event. We did not find any differences in tPA levels between the subgroups of NVAF patients; however, the small numbers in the subgroups may have limited the power of our study to detect any differences.

Our findings need to be confirmed in a prospective, longitudinal study. If the fibrinolytic system is found to

be activated in NVAF patients, our understanding of the mechanism of clot formation and stroke will be enhanced. The measurement of tPA levels may play a future role in decision-making regarding anticoagulation treatment for NVAF patients.

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