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Accumulation of risk factors enhances the prothrombotic state in atrial fibrillation ☆

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Abstract

Background: The present study was conducted to investigate the relation between the accumulation of the risk factors of thromboembolism and the levels of hemostatic markers in patients with nonvalvular atrial fibrillation (NVAF).
Methods: Five hundred ninety-one NVAF patients and 129 control subjects were categorized into low, moderate or high risk of thromboembolism, according to $CHADS_2$ index. One point each was given to patients with advanced age (≥75 years), hypertension, congestive heart failure, and diabetes mellitus, and 2 points, to those with prior ischemic stroke or transient ischemic attack. Patients with $CHADS_2$ score of 0, 1 or 2, and ≥3 were classified as low, moderate and high risk, respectively. Levels of hemostatic markers (platelet factor 4, β-thromboglobulin, prothrombin fragment F1+2 and D-dimer) were determined.
Results: Of 591 patients with NVAF, 302 were treated with warfarin (mean international normalized ratio 1.88). D-dimer levels increased as the risk level increased irrespective of warfarin use. Particularly, NVAF patients without receiving warfarin (n=289) had significantly higher D-dimer levels than control patients (e.g., for high risk patients, 175±144 vs 75±87 ng/ml, $p<0.001$), while NVAF patients receiving warfarin had intermediate levels (136±156 ng/ml). F1+2 levels increased as the risk level increased, and were significantly suppressed by warfarin. Levels of markers of platelet activation (platelet factor 4 and β-thromboglobulin) were increased in NVAF patients but not affected by the risk level.
Conclusion: Coagulation and fibrinolytic activity is increased along with the accumulation of the risk factors of thromboembolism in NVAF patients.

Keywords: Atrial fibrillation; Embolic risk; Hemostatic markers; Warfarin

1. Introduction

It is well established that patients with nonvalvular atrial fibrillation (NVAF) are at increased risk for thromboembolism, and anticoagulation with warfarin could decrease thromboembolic events by 61% but aspirin could do so by 19% [1]. Patients with NVAF could be in the prothrombotic state [2–6], especially when complicated with the well known risk factors for thromboembolism [7–12]. Patients with NVAF and elevated levels of D-dimer had higher

Abbreviations: AF, atrial fibrillation; F1+2, prothrombin fragment F1+2; INR, international normalized ratio; NVAF, nonvalvular atrial fibrillation; PF4, platelet factor 4; TIA, transient ischemic attack; β-TG, β-thromboglobulin.

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incidence of thromboembolic events [13]. Because accumulation of the risk factors quantified with $CHADS_2$ score was associated with an increase in the thromboembolic event [14,15], we hypothesized that accumulation of the risk factors could be related with increased levels of hemostatic markers. Therefore, we reanalyzed our previous data [12] plus additional groups of patients to investigate the relation between accumulation of the risk factors of thromboembolism and levels of hemostatic markers in patients with NVAF.

2. Materials and methods

2.1. Study subjects

A total of 591 patients (mean age 66.8±10.4 years, 65% men) including 380 patients with permanent atrial fibrillation (AF) and 211 patients with paroxysmal AF were enrolled in this study. One hundred twenty-nine age-matched patients in normal sinus rhythm were randomly selected from outpatients and served as control subjects. Five hundred nine NVAF patients and 111 control subjects were initially enrolled at 5 university hospitals for two years from September, 1999. Their clinical characteristics were reported elsewhere [12,13,15]. In the present study, 82 NVAF patients and 18 control subjects were added to the previous data [12]. The study protocol was approved by the ethics committee of each institution, and consent was obtained from each subject. Patients with mitral stenosis or mechanical heart valves were excluded. Chronic AF was confirmed electrocardiographically on at least two separate occasions (4 weeks apart). Paroxysmal AF was defined as AF documented electrocardiographically at least once in the preceding 12 months and lasting at least 1 h. The choice of antithrombotic therapy was left to the decision of each physician. Three hundred two patients received oral anticoagulation with mean international normalized ratio (INR) being 1.88±0.7 (SD) at the time of enrollment, and 179 patients, antiplatelet therapy with aspirin or ticlopidine, but 110 patients did not receive any antithrombotic therapy. Two hundred eighty-nine patients without receiving anticoagulation constituted the non-warfarin group, since antiplatelet therapy could not affect the levels of hemostatic markers [8,16–19].

2.2. Estimation of the risk for thromboembolism

Accumulation of risk factors for thromboembolism was calculated according to the $CHADS_2$ index [14]. That is, 1 point each was added for the presence of recent congestive heart failure, hypertension, age 75 years or older and diabetes mellitus, and 2 points, for history of stroke or transient ischemic attack (TIA). Five hundred ninety-one NVAF patients were classified into the two groups, i.e., NVAF with warfarin and NVAF without warfarin. In addition, subjects with sinus rhythm constituted the control group. Those with $CHADS_2$ score of 0 were classified as low risk, those with the score of 1 or 2, as moderate risk, and those with the score of ≥3, as high risk [15].

2.3. Blood sample collection and determination of hemostatic marker levels

Blood sample was obtained within 2 months from the enrollment in the study [12,20]. Levels of fibrin D-dimer (normal value<150 ng/ml) as a marker of fibrinolysis, prothrombin fragment F1+2 (F1+2, <1.4 nmol/l) as a marker of thrombin formation, and β-thromboglobulin (β-TG, <50 ng/ml) and platelet factor 4 (PF4, <20 ng/ml) as markers of platelet activation were determined at the central laboratory. Blood was taken from the antecubital vein using the two-syringe technique as described previously [12,20]. The first 2 to 3 ml of blood was discarded, and the subsequent samples were collected directly into syringes in a sequential manner. For determination of F1+2 and D-dimer, 0.2 ml of trisodium citrate was added to 1.8 ml of blood. Centrifugation was carried out within 2 h at 3000 rpm for 10 min at 4 °C. The anticoagulant mixtures for PF4 and β-TG contained theophylline, adenosine, dipyridamole, and sodium citrate. Mixtures of 2.7 ml of blood and anticoagulants were centrifuged at 3000 rpm for 30 min at 4 °C. Supernatant plasma was separated immediately and frozen rapidly at −20 °C for less than 24 h and subsequently stored at −80 °C until assayed. Levels of F1+2 and D-dimer were measured with enzyme-linked immunosorbent assay kits (Behring Werke AG, Marburg, Germany). PF4 and β-TG were measured with immunoassay kits (Behring Werke). Intra- and inter-assay coefficients of variation were 1.32 and 3.14% for D-dimer; 2.26 and 5.18% for F1+2; 3.62 and 4.96% for PF4; 4.73 and 7.43% for β-TG. An INR was also measured at each blood sample.

2.4. Analyses of data

The data are expressed as mean±SD. Comparison of continuous variables was performed with Kruskal–Wallis test for non-parametric distributions, followed by Dunn's method for

Table 1
Clinical characteristics of patients with nonvalvular AF and control subjects

	Nonvalvular AF		Control	*p* value
	Warfarin group	Non-warfarin group		
	(*n*=302)	(*n*=289)	(*n*=129)	
Men	201 (66.6)	186 (64.1)	80 (62.3)	0.59
Age (years)	67.2±9.0	66.4±11.6	67.0±10.4	0.81
Paroxysmal AF	78 (25.8)	133 (45.9)[a]	0[a,b]	<0.01
Hypertension	142 (47.0)	117 (40.3)	81 (62.3)[a,b]	<0.01
Diabetes mellitus	52 (17.2)	41 (14.1)	22 (16.9)	0.60
Hyperlipidemia	75 (24.8)	63 (21.7)	27 (20.8)	0.56
NYHA ≧2	89 (29.5)	50 (17.2)[a]	9 (6.9)[a,b]	<0.01
Prior stroke or TIA	81 (26.8)	40 (13.8)[a]	14 (10.8)[a]	<0.01

Values are number of patients (%) or mean±SD. AF = atrial fibrillation, NYHA = New York Heart Association functional class, TIA = transient ischemic attack.
[a] $p<0.05$ vs warfarin group.
[b] $p<0.05$ vs non-warfarin group.