CHEST

Original Research

ATRIAL FIBRILLATION

Soluble E-selectin, von Willebrand Factor, Soluble Thrombomodulin, and Total Body Nitrate/Nitrite Product as Indices of Endothelial Damage/Dysfunction in Paroxysmal, Persistent, and Permanent Atrial Fibrillation*

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Background: **Atrial fibrillation (AF) is associated with a prothrombotic state, which is related to endothelial damage/dysfunction. Plasma levels of soluble E-selectin (sE-sel), von Willebrand factor (vWf), and soluble thrombomodulin (sTM) have been used as indexes of endothelial activation, damage/dysfunction, and endothelial damage, respectively. Nitric oxide is also made by a healthy endothelium, and a total body nitrate/nitrite product (NOx) is used as a measure of endothelial nitric oxide production. We hypothesized that the levels of these markers of endothelial function would be abnormal in patients with paroxysmal, persistent, and permanent AF.**

Methods: **We studied 145 AF patients (paroxysmal AF, 35 patients; permanent AF, 50 patients; persistent AF, 60 patients) and 35 patients with "lone" AF. Plasma levels of sE-sel, vWf, and sTM (measured by enzyme-linked immunosorbent assay) and NOx (measured by a colorimetric assay based on the Griess reaction) were compared to 40 age-matched healthy control subjects in sinus rhythm.**

Results: **Patients with AF had significantly higher plasma levels of vWf ($p < 0.001$) and sE-sel ($p = 0.005$) compared with control subjects, but sTM and NOx levels were not significantly different. Levels did not differ significantly among the clinical subgroups of patients with paroxysmal, persistent, and permanent AF. Patients with lone AF had significantly higher vWF levels ($p = 0.003$) and significantly lower sTM levels ($p = 0.0361$) compared to control subjects, but sE-sel and NOx levels were not significantly different. There were no significant differences in the AF study population in vWF, sE-sel or sTM levels after 4 weeks of warfarin treatment.**

Conclusion: **Endothelial perturbation exists in all clinical subgroups of patients with AF, including those with lone AF, which may contribute to the prothrombotic state seen in these patients.** ***(CHEST 2007; 132:1253–1258)***

Key words: cardiology; coagulation; thrombosis

Abbreviations: AF = atrial fibrillation; BMI = body mass index; ELISA = enzyme-linked immunosorbent assay; INR = international normalized ratio; NOx = nitrate/nitrite product; sE-sel = soluble E-selectin; sTM = soluble thrombomodulin; vWf = von Willebrand factor

Atrial fibrillation (AF) is associated with an increased thromboembolic risk and a prothrombotic state.[1] Endothelial damage/dysfunction is a contributory factor to the prothrombotic state seen in many cardiovascular disorders, including AF.[1,2] Indeed, endothelial damage/dysfunction has been identified in many disease states and has been associated with worse outcomes and cardiovascular risk in patients with hypertension, atherosclerosis, diabetes mellitus, and many other conditions.[2] While the data in AF patients are more limited, plasma levels of von Willebrand factor (vWF), which is a well-established index of endothelial damage/dysfunction, have previously been shown to be consistently raised in AF patients, and related to stroke risk factors and prognosis.[1,3,4] Apart from vWf, there are many ways of assessing endothelial damage/dysfunction. Other plasma indexes of endothelial dysfunction variably reported in AF have included soluble thrombomodulin (sTM),[5] nitrate/nitrite product (NOx),[6] and impairment of forearm blood flow as measured by ultrasonography or plethysmography.[7]

We hypothesized that endothelial damage/dysfunction may contribute to the prothrombotic state in patients with AF, and that indexes of endothelial function (and dysfunction) may differ in the different clinical subgroups of AF. We also hypothesized that these endothelial indexes would be abnormal in patients with "lone" AF and altered after 4 weeks of anticoagulation therapy. To test these hypotheses, we conducted a cross-sectional and longitudinal study in which we measured plasma markers thought to originate from the endothelium in its different pathophysiologic states, in a large cohort of AF patients. Plasma vWF was measured as our "gold-standard" marker, alongside the following other endothelial plasma markers: (1) soluble E-selectin (sE-sel), which is specific to the endothelium and is thought to be released on endothelial activation[8]; (2) sTM, which is also specific to the endothelium and is used as an indicator of endothelial damage[9]; and (3) NOx as an indicator of total body nitric oxide production, the majority of which in the resting, fasted state is thought to be produced by endothelial nitric oxide synthase and is reduced in many conditions in which there is endothelial dysfunction.[10,11]

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Materials and Methods

We recruited 145 outpatients with nonvalvular AF during an 18-month period, as well as 35 subjects with lone AF. *Paroxysmal AF* was defined as at least two electrocardiographically documented episodes of AF with interceding sinus rhythm. *Persistent AF* was defined as AF lasting > 48 h without spontaneous reversion to sinus rhythm, suitable for and requiring pharmacologic or direct-current cardioversion to restore sinus rhythm. *Permanent AF* was defined as AF lasting for > 1 year in a patient in whom cardioversion was considered to be inappropriate or had previously been unsuccessful. Patients with AF who were receiving no antithrombotic therapy at baseline and had no associated risk factors such as hypertension, diabetes mellitus, ischemic heart disease, cardiac failure, or left ventricular hypertrophy (on ECG) were classified as having *lone AF*. We also recruited 30 patients with AF who were not yet receiving any antithrombotic therapy and who required anticoagulation (before undergoing elective direct-current cardioversion in either case) or who had at least one other risk factor for stroke and were in a moderate/high risk category in regard to risk stratification criteria. In this longitudinal study, venous blood samples were obtained at baseline, then at 4 weeks after the commencement of warfarin treatment, and after at least 3 weeks of therapeutic anticoagulation with a target international normalized ratio (INR) of 2 to 3. Patients were contacted during this period to ensure that the rapid establishment and maintenance of therapeutic anticoagulation had been achieved before the second visit. Any other changes to medication were avoided during this time.

Exclusion criteria were postoperative pneumonia or thyrotoxicosis, other infections, acute cardiovascular or cerebrovascular events (*eg*, myocardial infarction, stroke, or congestive heart failure) within 1 month, significant valvular heart disease, malignancy, connective tissue or inflammatory disease, chronic infection, and hepatic or renal impairment. AF patients were compared to a group of 40 age-matched healthy control subjects whose hearts were in sinus rhythm. The healthy control subjects were recruited from hospital staff and the relatives or friends of patients who attended hospital. Healthy control subjects had no history of diabetes mellitus, hypertension, neoplastic, connective tissue or cardiovascular disease, and all subjects underwent careful screening with baseline blood analysis, BP measurement, ECG, and echocardiography. The study was conducted in accordance with the Declaration of Helsinki following the approval of the West Birmingham Ethics Committee, and written informed consent was obtained from all participants.

Laboratory Analysis

Venous blood was obtained from subjects in the fasted state and > 8 h following the administration of any medication. Samples were collected into tubes (Vacutainer tubes; Becton Dickinson; Franklin Lakes, NJ) preloaded with sodium citrate (0.1 mmol/L) and stored on ice for a maximum of 20 min before processing. Platelet-poor citrated plasma was obtained from venous blood by centrifugation at 3000 revolutions per minute for 20 min at 4°C. Plasma was then aliquoted and frozen at −70°C for subsequent batch analysis.

Plasma vWf (in international units per deciliter) was measured using enzyme-linked immunosorbent assay (ELISA) [Dako;