Gender Differences in Fibrin Polymerization and Lysability of Fibrin in Patients with Atrial Fibrillation

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Background: Atrial fibrillation (AF) is the most common cardiac arrhythmia for both men and women. The embolic cardiovascular events represent serious complications of AF, and apparently women are affected more seriously than men. Little is known about prothrombotic factors and possible gender differences. The present study aimed to characterize fibrin polymerization, fibrinolysis, and fibrin fiber properties in men and in women with AF. *Materials and Methods:* Forty-six female and 101 male patients with AF and without previous stroke were included. Polymerization kinetics, lysis of preformed clot, and fibrin fiber properties were determined by turbidimetric methods. *Results:* Women were slightly older than men ($P < .01$), and the male group had a higher systolic blood pressure ($P < .01$) and a higher incidence of peripheral arterial disease ($P < .01$) than the female group. Compared with men, women had a higher V_{max} during fibrin polymerization ($P < .04$) and a lower lysability of fibrin, when recombinant tissue plasminogen activator (rt-PA) was added during clot formation ($P < .01$), while external lysis (rt-PA added after clot formation), plasma fibrinolytic activity, d-dimer, and fibrin fiber properties did not differ between men and women. A significantly higher number of men received acetylsalicylic acid (ASA) compared with women ($P < .004$). Subgroup analyses on subjects not receiving ASA demonstrated that women still had higher V_{max} ($P < .04$) and a lower rt-PA-induced fibrinolysis ($P < .03$). *Conclusion:* Women with AF have a higher velocity of lateral aggregation of fibrin fiber protofibrils and a lower lysis of fibrin clots than men. **Key Words:** Gender—fibrin structure—atrial fibrillation—cardiovascular disease.

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia for both men and women. The incidence increases dramatically with age, and the lifetime risk of AF in patients above 40 years of age is close to 25%.[1] The condition is associated with an increased risk of stroke, peripheral embolism, and death.[2-4]

In patients with AF, it has repeatedly been reported that women have an excess risk of embolic cardiovascular events compared with men. Wolf et al[3] originally presented their results from a large prospective study suggesting that female patients with AF had a significantly higher stroke risk. Others reported that in patients with AF and ischemic stroke, female gender per se predicted a poor outcome.[5] A number of subsequent studies and meta-analyses[5-9] acknowledge that female gender is a consistent risk factor, and female gender was proposed to be included in risk scoring according to the Birmingham 2009 scheme, that is, the CHA_2DS_2-VASc score.[10]

Although, the excess mortality in women with AF could be attributed to a higher degree of comorbidity, such as hypertension, hyperthyroidism, diabetes, or lower quality

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of life, the biochemical background is poorly elucidated. However, it is generally accepted that a prothrombotic state, a chronic low-grade inflammatory state, and endothelial dysfunction are associated with AF,[10-13] but it is largely unknown whether these are affected by gender.

Biomarkers are widely used in investigations of these conditions. Regarding the prothrombotic state, it seems relevant to study fibrin clot structure and the lysability of fibrin. It is unknown whether fibrin fiber characteristics differed between men and women in a healthy population, but a recent study reported that women with type II diabetes have more compact clots and prolonged lysis of clots than men with type II diabetics.[14] Patients with cardiovascular disease produce fibrin clots that are tighter and more rigid than clots from control subjects.[15-18] Such fibrin structure is more resistant to fibrinolysis and may therefore contribute to the pathogenesis of the thrombotic component of cardiovascular disease.[15-18]

The aim of the present study was to investigate the differences between men and women with AF with respect to a prothrombotic condition, with particular emphasis on fibrin polymerization kinetics, fibrin structure, and fibrinolysis.

Materials and Methods

Patients

The study participants were recruited from a cohort of consecutive patients in the maintenance phase of treatment with vitamin K antagonists (VKAs), at a specialized hospital-based anticoagulant clinic. The inclusion process has been described previously.[19] Briefly, 308 consecutive patients underwent a comprehensive interview followed by blood sampling at the anticoagulant clinic at the Department of Clinical Biochemistry, Hospital of South West Jutland, Denmark, between May 2009 and May 2010. The only exclusion criteria were age below 18 years and cancer chemotherapy.

Out of this cohort, 184 patients had a diagnosis of AF and 179 patients had availability of a full data set. A total of 32 AF patients had a diagnosis of stroke or transient ischemic attack and were excluded from the present analysis.

The declaration of Helsinki was followed throughout the study and approval was obtained from the regional Ethics Committee (project ID S-20080020).

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Collection of Blood

Samples of blood were collected in evacuated siliconized coated tubes containing sodium citrate (4.5 mL blood, .5 mL .109 mol/L trisodium citrate dihydrate). Platelet poor plasma was prepared by centrifugation at 2000 *g* for 20 minutes within 1 hour after sampling. Specimens of 350 µL plasma were stored at −80°C until analysis.

Biochemical Analysis

Properties of fibrin clots were studied with turbidity measurements.[18,19] The polymerization of fibrin was studied by mixing 60 µL of plasma with 120 µL of a reaction mixture containing bovine thrombin (1 IU/mL), calcium (15 mmol/L), Tris–HCl (50 mmol/L), and NaCl (150 mmol/L). OD_{340} was recorded for 30 minutes. V_{max}, peak optical density (OD), and overall coagulation potential were calculated as previously reported.[18,19]

The lysability of fibrin was determined by a similar setup in another clot with the following modification: recombinant tissue plasminogen activator (rt-PA) Actilyse; Boehringer Ingelheim, Ingelheim am Rhein, Germany was added to the reaction mixture in a concentration of 300 ng/mL and OD_{340} was recorded for 30 minutes. Lysis of clot, overall hemostasis potential, and overall fibrinolysis potential (OFP) were calculated as previously reported.[18,20,21]

The lysability of fibrin on a preformed clot was determined in a similar setup with the following modification: 60 µL of a separate lysis buffer containing 50 µg/mL rt-PA was added after clot formation, and OD_{340} was recorded for 240 minutes.

Fibrin fiber properties were determined by measurement of the $OD_{405,540,608,690}$ of the fibrin clot and subsequent calculation of the fiber mass/length ratio, fiber diameter, and fiber mass density.[18,21]

Global fibrinolytic activity of plasma was determined by a fibrin plate assay.[22] The fibrinolytic activity was calibrated against the 3rd International Standard for recombinant t-PA (NIBSC 98/714), and the results expressed in international unit per milliliter.

Plasminogen activator inhibitor-1 (PAI-1) protein concentration was determined with an ELISA method in microtiter plates, with a kit from Trinity Biotech (Bray, Ireland).[23] The concentrations of fibrin d-dimer and fibrinogen were determined on an automated analyzer (STA-R; Diagnostica Stago, Asnières-Sur-Seine, France). The concentration of C-reactive protein was determined on a nephelometer (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). The prothrombin time was determined with the use of reagents from Diagnostica Stago on a STA-R analyzer and expressed in international normalized ratio (INR).

Statistical Analysis

The Mann–Whitney *U*-test and the Fisher exact test were used to compare groups. The Spearman rank-order correlation test was used to test for associations. A *P* value less than .05 was considered significant. Data are presented as median and quartiles or number and percentages.