A Study of blood soluble P-selectin, fibrinogen, and von Willebrand factor levels in idiopathic and lone atrial fibrillation

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Aims
A prothrombotic state with elevated levels of soluble P-selectin (sP-sel), fibrinogen, von Willebrand factor (vWF), and other haemostatic indices has been reported in some patients with atrial fibrillation (AF). Whether these changes are due to AF itself or coexistent cardiovascular diseases remains a matter of debate. Therefore, in the present study, the differences in plasma levels of sP-sel, fibrinogen, and vWF between patients with idiopathic/lone AF and sex-, age-, and risk factor-matched controls were investigated to determine whether AF itself might be associated with a hypercoagulable state.

Methods and results
Ninety consecutive patients (63 males, 54.1 ± 10.1 years) with idiopathic AF were studied, 60 (43 males, 48.8 ± 7.5 years) of whom were diagnosed as lone AF. Plasma sP-sel and vWF were measured by enzyme-linked immunosorbent assay. Plasma fibrinogen was measured by chromometry. These indices in AF patients were compared with those in sex-, age- and risk factor-matched controls.

Compared with the controls, patients with idiopathic AF had higher levels of sP-sel (AF vs. control: 33.4 ± 7.4 vs. 29.2 ± 6.5 ng/mL, P = 0.001) and fibrinogen (AF vs. control: 3.3 ± 0.9 vs. 3.0 ± 0.6 g/L, P = 0.02), but not vWF, whether with the adjustment of covariates or not. As for those <60 years, between lone AF and age-matched controls, significant difference existed in the levels of sP-sel (AF vs. control: 34.5 ± 7.3 vs. 30.2 ± 7.3 ng/mL, P = 0.002), but not in those of fibrinogen and vWF, whether with the adjustment of covariates or not.

Conclusions
Both platelet activation and abnormal changes in coagulation were suggested in idiopathic AF and a platelet activation state in lone AF. This supports the notion that AF per se contributes to a state of hypercoagulation.

Keywords
Atrial fibrillation • Soluble P-selectin • Fibrinogen • von Willebrand factor

Introduction
Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, is a major cause of morbidity and mortality through an increased risk of thrombo-embolic stroke.1,2 The mechanisms behind cerebral thrombo-embolism in AF are incompletely understood, but it is well documented that AF is associated with a prothrombotic state as demonstrated by higher levels of plasma soluble P-selectin (sP-sel; a marker of platelet activation), fibrinogen (the precursor to insoluble fibrin and an important rheological factor), von Willebrand factor (vWF; a marker of endothelial damage/dysfunction), and other haemostatic indices, when compared with healthy control subjects3–7 leading to suggestions of a generalized prothrombotic state in AF.

However, since most of the studies are confounded by differences in baseline characteristics such as age, gender, other risk factors, and especially cardiovascular disease status,3,6,8–13 which are known to influence haemostatic factor levels,10,11,14–17 whether these changes of thrombogenic indices are due to AF itself or confounding disorders remains debatable.3,10,13,18–20 Because of the additional thrombo-embolic risk posed by AF itself,1,2 we hypothesized that AF itself was associated with the
observed changes in prothrombotic markers. Therefore, levels of sP-sel, fibrinogen, and vWF in idiopathic and lone AF were compared with those in sex-, age-, and risk factor-matched controls so as to determine whether AF itself is associated with a hypercoagulable state or not.

**Methods**

**Study population**

The study was approved by the Ethics Committee of Guangdong Provincial Hospital, Guangzhou, P.R. China, and written informed consent was obtained from each participant.

In the present study, AF was diagnosed by electrocardiogram (ECG) recordings, including 12-lead surface ECGs and 24 h Holter recordings. It was also diagnosed by a medical history of AF, which was reported by physicians previously consulted. Idiopathic AF was defined as the AF in the absence of any defined causes of the arrhythmia (summarized in ‘exclusion criteria’ below). The term ‘lone AF’ had been variously defined but applied to young idiopathic AF (under 60 years of age) in this study. All patients with a diagnosis of AF, who visited Guangdong Provincial Hospital, Guangzhou, from April 2007 to July 2009, were scanned and 90 met the criteria of idiopathic AF and were prospectively enrolled to constitute the patient group, 60 in which were diagnosed as lone AF. The control group included 79 sex-, age-, and risk factor-matched individuals who were recruited from people visiting the general health check-up division at the hospital. They did not take regular medications except for statins and had no evidence of diseases mentioned in the exclusion criteria on careful history, clinical examination, and basic blood and instrument tests.

Exclusion criteria include >75 years old, overt atherosclerosis (carotid artery sclerosis in vascular ultrasound or aortosclerosis in chest X-ray), hypertension, coronary heart disease, congenital heart disease, severe valve disease (subjects with mitral regurgitation were randomly enrolled into the study), myocardiopathy, heart failure, diabetes mellitus, malignancies, chronic inflammatory diseases, hyperthyroidism, hepatic or renal failure, mental disorder, chronic alcohol abuse, and thrombo-embolism. Subjects with acute inflammatory diseases, surgery and trauma within 60 days, as well as AF secondary to hypoxaemia, drugs abuse, and acute alcohol intoxication were also excluded.

**Blood collection and laboratory analysis**

Each subject provided a detailed medical history and underwent a physical examination, chest X-ray, bilateral carotid arteries sonography, and transthoracic echocardiogram. In addition to the history of cardiovascular disease, the following risk factors were also recorded: a history of hyperlipidaemia and cigarette smoking. The latter referred to former and current smokers.

Blood samples were obtained from a peripheral vein in the morning after an overnight fasting period. Platelet counts were determined from whole blood utilizing standard laboratory methods. Plasma fibrinogen levels were measured by using a clot-based turbidometric detection system within 2 h of phlebotomy. Blood samples for vWF and sP-sel assays were drawn into 3.8% sodium citrate tubes, immediately mixed by gentle inversion, stored on melting ice, and centrifuged at 4°C for 1790g x 5 min within 1 h of phlebotomy, and then plasma was separated into aliquots and stored at −70°C to allow batch analysis for sP-sel and vWF assays. Measurements of sP-sel and vWF were performed using enzyme-linked immunosorbent assay (ELISA) with reagents from R&D Systems (Abington, UK) and Dako-Patts (Ely, UK), respectively. The unit for vWF is IU/dL and was standardized by reference vWF from the National Institute for Biological Standards and Controls (Potters Bar, Hertfordshire, UK). Intra-assay coefficients of variation for all ELISAs were <5%, and inter-assay variances were <10%.

**Power calculation and data analysis**

We hypothesized that patients with AF would have levels of fibrinogen ~0.25 of a standard deviation higher than those of subjects in sinus rhythm. To achieve this with a 1 – β power of 0.80 and P < 0.05, 40 subjects per group are required. In view of our measurement of multiple indexes and to minimize the risk of a type II error, we recruited in excess of this number of AF patients.

Categorical variables were presented as frequencies (percentage) and compared using Pearson’s χ² test. Continuous data were expressed as mean ± SD. Because of skewed distribution, platelet counts, plasma levels of fibrinogen, sP-sel, and vWF were all analysed after logarithmic transformation. t tests were performed to compare continuous variables between two groups. Differences in circulating sP-sel, fibrinogen, and vWF levels between AF patients and controls were also analysed by analysis of covariance (ANCOVA), with age, sex, and a history of hyperlipidaemia, smoking, diastolic blood pressure, and systolic blood pressure as the covariates for fibrinogen and vWF, and the above covariates and platelet counts for sP-sel, as these were factors that might have influence on these indices.

Subgroup analysis according to age stratification was also performed. SPSS 13.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis.

**Results**

A total of 169 individuals, including 90 idiopathic AF patients (72 paroxysmal, 13 persistent, and 5 permanent AF patients, according to the definition of AF in the ACC/AHA/ESC 2006 AF guideline) and 79 sex-, age-, and risk factor-matched controls, were studied. Since persistent or permanent AF patients tend to be complicated by heart diseases or have a large left atrium size both of which meet the exclusion criteria of this study, only few persistent and permanent AF patients were enrolled. Clinical trial data suggest that paroxysmal AF confers similar risk of stroke similar to persistent or permanent AF. In this study, sP-sel, fibrinogen, and vWF levels showed no differences between paroxysmal and non-paroxysmal AF patients. Thus, data from these three different types of AF were pooled for analysis in this study.

Basic characteristics and research indices are summarized in Table 1. No significant differences in sex, age, and a history of hyperlipidaemia and smoking, systolic blood pressure, diastolic blood pressure, platelet counts, and plasma levels of vWF were found between the idiopathic AF cases and controls.

Compared with control subjects, individuals with idiopathic AF had higher levels of plasma sP-sel and fibrinogen. The differences remained statistically significant even after adjustment for the covariates listed by ANCOVA (Table 1).

As for 60 lone AF patients (48 paroxysmal, 8 persistent, and 4 permanent AF patients) and 50 age-matched controls, there are no significant differences in sex, age, and a history of...
hyperlipidaemia and smoking, systolic blood pressure, diastolic blood pressure, and platelet counts (Table 2).

Plasma levels of sP-sel but not those of plasma fibrinogen and vWf were higher in lone AF patients than in age-matched controls. The difference remained statistically significant even after adjustment for covariates listed by ANCOVA (Table 2).

**Discussion**

The main finding of the present study is that the strictly defined idiopathic AF patients had higher plasma levels of sP-sel and fibrinogen than the sex-, age-, and risk factor-matched controls. Although conflicting results have been showed in some
studies, elevations in sP-sel and fibrinogen have both been described in AF cohorts in most of the previous studies. Of note, the AF cohorts in these studies were mostly combined with the underlying cardiovascular diseases. This would certainly disturb the judgement of the association of thrombogenic indices with AF itself. As a result, it has been controversial whether the arrhythmia itself independently leads to platelet activation and abnormal changes of coagulation or not. In some studies, the higher levels of circulating fibrinogen were ascribed to AF per se, whereas in another study, these were presumed to be mainly due to the effect of the confounding traditional risk factors and coexistence of cardiovascular disease. Similarly, the elevation of sP-sel levels was ascribed to the underlying cardiovascular diseases rather than the AF per se in some studies, whereas a more recent study by Akar et al. showed that stimulated AF caused local cardiac platelet activation within minutes of AF onset.

Because the AF patients enrolled in the current study were free of the coexistence of cardiovascular diseases, the finding of higher levels of sP-sel and/or fibrinogen in the patients group should support the notion that AF alone, independent of other risk factors and cardiovascular diseases, may relate to platelet activation and abnormal changes of coagulation.

Up to date, only few studies have focused on the changes of thrombogenic indices in idiopathic AF patients. In the subgroup analysis of Framingham Offspring Study, AF without cardiovascular disease and controls without cardiovascular disease had no differences in the prothrombotic indices (including fibrinogen). However, Mondillo et al.’s study showed that plasma levels of fibrinogen, platelet factor 4, and thromboglobulin in the ‘lone’ AF patient (mean age 67.6 ± 8.3 years old) were higher than those in the controls, but with the changes of sP-sel level not involved. In addition, it did not investigate these differences according to the stratification of age, mainly due to the small sample size.

Because there is an age-related increase in the risk of stroke in non-valvular AF and idiopathic AF <60 years old is deemed very low risk of stroke, we further analysed the differences in the research indices between AF patients and controls in the subgroup <60 years old. Although variously defined, the term ‘lone AF’ has been generally applied to those AF patients without cardiovascular diseases and under 60 years of age.

In this study, plasma sP-sel but not fibrinogen was significantly higher in lone AF patients than that in age-matched controls. Thus, the current study should indicate that a state of platelet activation does exist in lone AF patients.

Although the importance of platelets in enhancing the thrombogenic tendency in AF was questioned in some study, plasma sP-sel, a well-established index of platelet activation, had previously been reported to be associated with intra-atrial echocardiography contrast and thrombus, and predictive of adverse clinical outcomes in AF patients. Thus, together with these studies, our results may be a useful clue for anti-platelet therapy for lone AF patients.

According to the ACC/AHA/ESC 2006 Guidelines for the management of patients with AF, aspirin (81–325 mg per day) or no therapy is recommended for preventing thrombo-embolism in lone AF patients. The results in this study suggest that anti-platelet therapy might be more reasonable than no therapy in this strictly defined population. This opinion is consistent with the more recent antithrombotic guidelines ACPP-8, in which all chronic AF patients are recommended at least long-term aspirin therapy.

Notably, no difference was found in plasma vWF between AF patients and controls, both in group analysis and subgroup analysis. Most of the relevant studies found that blood vWF was raised in patients with AF. Because the AF cohorts in these studies were mostly combined with cardiovascular disease, it remains a matter of debate whether the raised vWF levels are due to the AF itself or coexistent cardiovascular diseases. In the Framingham Offspring study, the difference in vWF levels between chronic AF and healthy controls was no longer significant when the patients were stratified according to other cardiovascular diseases and risk factors. In fact, although no significant difference exited in vWF between idiopathic AF patients <60 and those ≥60 years old (<60 vs. ≥60: 113.6 ± 34.1 vs. 122.4 ± 43.2 IU/dL, P = 0.298), it is clear from the descriptive data above that the ≥60-year-old AF patients had higher vWF levels. This implied old age may have an effect on the levels of vWF. However, the data previously shown by Mondillo et al. demonstrated that vWF levels were raised in their lone AF cohort.

In the previous study, increased vWF expression had been demonstrated by immunohistochemical means in endocardial cells from the atrial appendage of AF patients, this implied that the increase in vWF occurred on a cardiac level. As the increase in vWF in peripheral circulation probably occurred as a result of the increased local endothelial tissue vWF expression, vWF changes in plasma showed in the present study might not fully reflect those in atrium. This might partly account for non-significant difference in plasma vWF levels between idiopathic AF patients and controls in this study.

Limitations

There were some limitations in this study. First, this study was a relatively small sample study and this might potentially lead to spurious findings. For example, the power of the difference in fibrinogen between lone AF patients and age-matched controls is only 0.365. Would the sample size be larger, the difference in fibrinogen between lone AF patients and controls might have become significant. In addition, the use of ANCOVA (a type of regression analysis) might produce types 1 and 2 error resulting from insufficient sample size.

Secondly, although medical history and clinical examinations, including ECG and/or Holter recordings, were performed, the prevalence of AF might be underestimated in the controls group, because AF is sometimes characterized by the absence of symptoms.

Thirdly, although there was no obvious evidence for atherosclerosis by carotid artery sonography and chest X-ray in all subjects, the possibility of atherosclerosis could not be totally excluded.

In addition, some people were combined with hyperlipidaemia or cigarette smoking. Both are risk factors for atherosclerosis and known to influence haemostatic factor levels. However, since hyperlipidaemia and cigarette smoking were not the focuses of this study, we sought to match them between patient group and control group, to eliminate the disturbance.
There may be the potential for unmeasured differences (e.g. left atrium size and interventricular septum diameter, data not shown) between groups that may have affected the results. However, available evidence already pointed towards a lack of a significant relationship between markers of thrombogenesis and patients’ structural cardiac abnormalities on echocardiography.18,28,36,37 Furthermore, given that there was lack of associated heart diseases and that the left atrium size and interventricular septum diameter in AF patients were almost in normal range, we would rather think that AF caused the morphological changes than that the morphological changes caused AF.

The present study was also limited by warfarin or aspirin use in a few AF subjects, so it is not possible to completely eliminate the potential confounding influence of medications on biomarker levels. However, in some studies, aspirin therapy failed to reduce plasma indices of coagulation and platelet activation in AF.28,35 In addition, most of the past studies showed that the introduction of warfarin did not significantly alter the levels of fibrinogen.18,28,36,37 Although in one study, sP-sel was found to be elevated on warfarin therapy with the reason unclear,35 we could not presume that warfarin use in the patients group accounted for changes caused AF.

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