SCIENTIFIC LETTER

Platelet activation, haemorheology and thrombogenesis in acute atrial fibrillation: a comparison with permanent atrial fibrillation

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It has previously been believed that atrial thrombus, which is the source of thromboembolism in atrial fibrillation (AF), is formed after 48 hours of onset of AF, but there are limited clinical and pathophysiological data to confirm this convincingly.¹ Therefore, oral anticoagulation treatment is recommended before and after cardioversion of AF of more than two days' duration, to minimise thromboembolic complications.² In contrast, restoration of sinus rhythm in patients with AF lasting less than two days has previously been regarded as "safe", and prolonged anticoagulation treatment (except for heparin) was not thought to be mandatory.² Despite these guidelines, many instances of thromboembolism still occur in acute AF and a better understanding of the pathophysiological processes leading the thromboembolism in acute AF is needed. Indeed, conversion to sinus rhythm may increase the thrombogenic milieu of the left atrium.^{1,3} Furthermore, evidence that AF confers a hypercoagulable state is growing.⁴ However, it is unclear exactly how long after the commencement of AF this increased risk of thrombosis becomes apparent and therefore clinically important. For example, does it take hours, days or weeks for the hypercoagulable state to develop?

We hypothesised that platelet activation, haemorheology, and thrombogenesis occur within 48 hours of onset of AF, as a pathophysiological mechanism contributing to increased thromboembolism in this condition. To test this hypothesis, we measured indices of platelet activation (ex vivo platelet aggregation, as well as plasma soluble P-selectin and plasma β thromboglobulin (β -TG)), haemorheology (haematocrit and plasma viscosity), and thrombogenesis (fibrin D-dimer) in 31 patients with acute onset AF, who were compared with 93 patients with permanent AF and 31 healthy controls in sinus rhythm. Furthermore, 15 patients with acute AF from this same cohort were reanalysed for the above parameters after (spontaneous) reversion to sinus rhythm at outpatient follow up at two months.

PATIENTS AND METHOD

We recruited "acute" AF patients admitted with sudden onset of AF and within 48 hours after the onset. These patients therefore include patients with the first symptomatic onset of persistent AF and some with acute episodes of (previously known) paroxysmal AF. The duration of acute AF was estimated from the time of symptom onset to the time of blood sample, and all acute AF patients were still in AF at the time of sampling. For every patient in acute AF, we recruited three age (± 10 years) matched patients with permanent AF and one healthy control in sinus rhythm. The study group therefore comprised 31 patients with acute AF (19 males, mean (SD) age 61(17) years), who were matched with 93 patients with permanent AF (59 males, 66 (7) years) and 31 healthy controls in sinus rhythm (13 men, 66 (12) years). Patients who were uncertain of the time of onset, those who could not reliably recall the time of onset of arrhythmia, those who were

known to have an "irregular pulse", "AF" or other arrhythmia(s), and those who were already on warfarin or heparin were excluded. We also excluded any patients with systemic disorders that might influence research indices.

The "acute AF" patients who were recruited were subsequently reviewed in the hospital outpatient clinic at two months, and follow up blood samples were taken if they had spontaneously reverted to sinus rhythm following admission ($n = 15$). Patients who remained in AF at two months, those who had to be electrically or pharmacologically cardioverted to sinus rhythm since the onset of acute AF, and those who were commenced on warfarin or had their antithrombotic treatment altered (this would have been done if AF had persisted for > 48 hours), were excluded from the follow up study. Patients with permanent AF who were not on warfarin were recruited from referrals to a specialist AF clinic. Blood results in patients with AF were compared with "healthy controls", who comprised 31 healthy subjects in sinus rhythm, recruited from healthy hospital staff, relatives of the patients, and those attending the hospital for routine senile cataract surgery.

Blood samples (approximately 10 ml) were drawn from an antecubital vein with atraumatic venepuncture into plastic tubes with 3.2% sodium citrate or CTAD (citrate, theophylline, adenosine, and dipyridamole). Plasma was separated for the quantification of plasma soluble P-selectin, β -TG, fibrin D-dimer (all enzyme linked immunosorbent assay (ELISA)), and fibrinogen (Clauss technique). Citrated blood for platelet aggregation was maintained at room temperature (22°C) until sample preparation, to prevent in vitro platelet lysis or activation. Citrated blood tubes were centrifuged within one hour of collection, at room temperature at 150 g for 10 minutes. The supernatant, platelet rich plasma, was separated. The residual sample was then centrifuged at 2500 g for 10 minutes to obtain a clear citrated supernatant, of platelet-free plasma. Ex-vivo platelet aggregation was measured in a plasma platelet aggregometer (Platelet aggregation profiler, model PAP-4, Bio/data Corp, Horsham, Pennsylvania, USA). Aggregation was induced by the addition of the following reagents: ADP (adenosine diphosphate, final concentration of 0.1 μ mol/ml), collagen (final concentration of 2 mg/ml), adrenaline (epinephrine) (final concentration of 0.1 μ mol/ml) (Sigma Diagnostics, St Louis, Missouri, USA) and thrombin (final concentration of 1 NIH units/ml) (Fisher diagnostics, Pacific haemostasis, Huntersville, North Carolina, USA). Platelet aggregation curves were recorded and the extent of platelet aggregation was evaluated by measuring the percentage platelet aggregation at the end of three minutes.

Abbreviations: ADP, adenosine diphosphate; AF, atrial fibrillation; ANF, atrial natriuretic factor; ANOVA, analysis of variance; β -TG, plasma β thromboglobulin; CTAD, citrate, theophylline, adenosine, and dipyridamole; ELISA, enzyme linked immunosorbent assay

Table 1 Platelet activation, haemorheology, and thrombogenesis in patients with acute atrial fibrillation (AF), compared with permanent AF and healthy controls in sinus rhythm

	Acute AF	Permanent AF	Healthy controls	p Value†
Blood platelets ($\times 10^9/l$)	280 (81)	264 (75)	253 (51)	0.36
Hematocrit (%)	45 (4)*	43 (5)	42 (3)	0.03
Fibrinogen (g/l)	2.9 (0.7)	2.7 (0.6)	2.6 (0.4)	0.22
Plasma viscosity (mpa)	1.8 (0.1)	1.7 (0.1)	1.7 (0.1)	0.15
β -TG (IU/ml)	87 (59–110)	86 (58–121)	67 (50–90)	0.09
Soluble P-selectin (ng/ml)	38 (6)	39 (10)	36 (11)	0.30
Fibrin D-dimer (ng/ml)	710 (420–1200)	1100 (790–3000)**	580 (290–1100)	<0.001
Platelet aggregation in response to (agonist)				
ADP	72 (60–84)	66 (50–76)	70 (64–80)	0.054
Collagen	50 (20–70)	20 (08–56)	32 (10–60)	0.08
Adrenaline (epinephrine)	37 (27–49)	40 (22–56)	58 (18–74)	0.27
Thrombin	20 (03–50)	56 (12–79)	48 (03–80)	0.12

Results are expressed as mean (SD) or as median (interquartile range, IQR) for the normally distributed data and skewed data, respectively. Platelet aggregation expressed as median (IQR) percentage aggregation at 3 minutes.

Tukey's post-hoc analysis for inter-group comparisons: * $p < 0.05$ compared to healthy controls; ** $p < 0.001$ compared to healthy controls.

†One way ANOVA or Kruskal Wallis tests were used as appropriate, for comparison of the three groups.

β -TG, β thromboglobulin; ADP, adenosine diphosphate

Data were analysed by Kruskal-Wallis test or one way analysis of variance (ANOVA), with Tukey's post-hoc analysis (on logged data, where appropriate). Paired samples were analysed by paired t test or Wilcoxon test, as appropriate. Subgroups of acute AF patients were analysed by unpaired t test or Mann Whitney U test.

RESULTS

Blood platelet count, plasma fibrinogen, and viscosity values were similar in healthy controls and AF patients, whereas mean haematocrit concentrations were significantly higher in patients with acute AF compared to healthy controls (one way ANOVA, $p = 0.03$). Plasma concentrations of β -TG and soluble P-selectin were not significantly different. However, median plasma fibrin D-dimer values were significantly higher in the patient groups with AF (Kruskal-Wallis test, $p < 0.001$), with concentrations in permanent AF higher compared to healthy controls (Tukey's test, $p < 0.05$) but not significantly different between patients with acute AF and healthy controls. Platelet aggregation parameters in patients with AF (acute or chronic) were similar to healthy controls in response to all four platelet agonists (table 1). Demographic details of patients and controls are summarised in appendix table 1.

In a cohort of 15 eligible patients (10 males, 61 (18) years, range 24–85 years) there were no significant differences in indices of platelet activation, haemorheology, and thrombogenesis in the "acute AF" patients while in AF and when in sinus rhythm (appendix tables 2 and 3). When patients with acute AF were further analysed depending on the duration of the arrhythmia (group 1 (duration of AF < 12 hours) and group 2 (> 12 hours); appendix table 4), most of the indices of platelet activation, haemorheology, and thrombogenesis were similar between the two, except for plasma fibrinogen values, which were significantly higher in group 1 patients ($p = 0.02$) (appendix table 4). Prior aspirin treatment did not make any significant difference to the various parameters in acute AF patients (data not shown). In the whole cohort of AF patients (both acute and permanent), higher median fibrin D-dimer concentrations were found in the patients taking aspirin ($p = 0.04$), but this subgroup was also older ($p = 0.01$), while platelet aggregation to thrombin was lower in patients taking aspirin ($p = 0.04$) (appendix table 5).

DISCUSSION

This study extends our appreciation of possible differences in the hypercoagulable state associated with subgroups of AF,⁵

demonstrating that patients with acute AF have a high haematocrit, but not platelet activation or thrombogenesis (fibrin D-dimer) when compared to controls in sinus rhythm. Previous studies have demonstrated that haematocrit increases within a few hours of onset of paroxysmal AF,^{6–9} remaining high even up to four years,⁹ providing evidence for haemoconcentration in AF.

The high haematocrit observed with acute AF could be related to some haemoconcentration secondary to high filling pressures in the left atrium with the onset of AF stimulating the release of atrial natriuretic factor (ANF) pro-hormone, proANF.^{10–12} A raised haematocrit is more prevalent in patients with left atrial spontaneous echo contrast¹³ and is thought to potentially predict recurrent thromboembolism¹⁴ and mortality in patients with acute stroke.¹⁵ However, in our study, raised haematocrit values were not obvious in patients with permanent AF. One reason for this could perhaps be related to degenerative changes in the left atria in AF, resulting in an insufficiency in the production of ANF.¹⁶

Contrary to the present study, increased concentrations of plasma β -TG have been reported in patients with AF.⁴ However, it is possible that platelet activation does not appear to play an important role in the stroke and thromboembolic risk in patients with AF (as reviewed by Kamath and colleagues¹⁷). Perhaps what matters in AF is activation of the coagulation system rather than platelet activation; indeed, stroke and thromboembolism in AF are thought to be caused by fibrin-rich atrial thrombi, rather than platelet-rich thrombi. For example, fibrin D-dimer is higher in AF patients with stroke than those without stroke.¹⁸ In the present study, we found significantly higher fibrin D-dimer concentrations in patients with permanent AF but not in acute AF, when compared to healthy controls. This further supports the current concept that acute AF may not predispose significantly to stroke and thromboembolism for the first 48 hours, whereas permanent AF significantly predisposes to thromboembolism.²

It is interesting that there were no differences in any of the haematological and platelet parameters between episodes of acute AF and subsequent sinus rhythm. This may very well signify that the level of coagulation system activation and platelet activation during acute AF was "comparable" to that seen in sinus rhythm, and therefore fails to impose any significant thromboembolic risk. Furthermore, we did not find any significant difference in the plasma concentrations of fibrin D-dimer or markers of platelet activation (including platelet aggregation) between acute AF patients where the arrhythmia lasted shorter or longer than 12 hours. This may

imply that the "low" level of thromboembolic risk may last as long as 48 hours after onset of AF, thus supporting the current practice of safe cardioversion within 48 hours of onset of AF without resorting to prolonged anticoagulation before and after cardioversion.² Nevertheless, higher plasma fibrinogen concentrations were seen in the patients with acute AF of < 12 hours duration, suggesting early haemorheological abnormalities and the development of spontaneous echo-contrast following AF onset would be consistent with this.¹³

In conclusion, patients with acute AF have a higher haematocrit than healthy controls. However, increased thrombogenesis was only noted in permanent AF, contributing to the increased thromboembolic risk and the need for anticoagulation in these patients.

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REFERENCES

- 1 Stoddard MF. Risk of thromboembolism in acute atrial fibrillation or atrial flutter. *Echocardiography* 2000;17:393–405.
- 2 Lip GYH, Hart RG, Conway DSG. ABC of antithrombotic therapy. Antithrombotic therapy for atrial fibrillation. *BMJ* 2002;325:1022–5.
- 3 Oltrona L, Broccolino M, Merlini PA, et al. Activation of the hemostatic mechanism after pharmacological cardioversion of acute nonvalvular atrial fibrillation. *Circulation* 1997;95:2003–6.
- 4 Lip GYH. Does atrial fibrillation confer a hypercoagulable state? *Lancet* 1995;346:1313–4.
- 5 Li-Saw-Hee FL, Blann AD, Gurney D, et al. Plasma von Willebrand factor, fibrinogen and soluble P-selectin levels in paroxysmal, persistent and permanent atrial fibrillation. Effects of cardioversion and return of left atrial function. *Eur Heart J* 2001;22:1741–7.
- 6 Sahara H, Miyahara K. Effect of atrial fibrillation on the fibrino-coagulation system – study in patients with paroxysmal atrial fibrillation. *Jpn Circ J* 1994;58:821–6.
- 7 Takahashi N, Ashida T, Kiraku J, et al. Increase in erythrocyte volume in patients with chronic atrial fibrillation. *Jpn Heart J* 1997;38:387–91.
- 8 Okuno S, Ashida T, Ebihara A, et al. Distinct increase in hematocrit associated with paroxysm of atrial fibrillation. *Jpn Heart J* 2000;41:617–22.
- 9 Yamada H, Sugiyama T, Ashida T, et al. Sustained hemoconcentration in patients with chronic atrial fibrillation, a potential risk for stroke and thromboembolic complications. A retrospective study. *Jpn Heart J* 1998;39:715–20.
- 10 Horneftam B, Hall C, Held P, et al. N-terminal proANF in acute atrial fibrillation: a biochemical marker of atrial pressures but not a predictor for conversion to sinus rhythm. Digitalis in acute atrial fibrillation (DAAF) trial group. *Am Heart J* 1998;135:1040–7.
- 11 Roy D, Paillard F, Cassidy D, et al. Atrial natriuretic factor during atrial fibrillation and supraventricular tachycardia. *J Am Coll Cardiol* 1987;9:509–14.
- 12 Petersen P, Kastrup J, Vilhelmsen R, et al. Atrial natriuretic peptide in atrial fibrillation before and after electrical cardioversion therapy. *Eur Heart J* 1988;9:639–41.
- 13 Hwang JJ, Ko FN, Li YH, et al. Clinical implications and factors related to left atrial spontaneous echo contrast in chronic nonvalvular atrial fibrillation. *Cardiology* 1994;85:69–75.
- 14 Yasaka M, Yamaguchi T, Oita J, et al. Clinical features of recurrent embolization in acute cardioembolic stroke. *Stroke* 1993;24:1681–5.
- 15 Lowe GD, Jaap AJ, Forbes CD. Relation of atrial fibrillation and high haematocrit to mortality in acute stroke. *Lancet* 1983;i:784–6.
- 16 Van Den Berg MP, Crijns HJ, Van Veldhuisen DJ, et al. Atrial natriuretic peptide in patients with heart failure and chronic atrial fibrillation: role of duration of atrial fibrillation. *Am Heart J* 1998;135:242–4.
- 17 Kamath S, Blann AD, Lip GYH. Platelet activation in atrial fibrillation. *Eur Heart J* 2001;22:2233–42.
- 18 Ono N, Koyama T, Suehiro A, et al. Influence of atrial fibrillation on coagulo-fibrinolytic markers in patients with cerebral infarction. *Int Angiol* 1992;11:298–303.

To see appendix tables 1–5 go to the *Heart* website—www.heartjnl.com/supplemental

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Controlling CVD in older British women spells a heavy workload

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A survey of more than 4000 older British women has concluded that appreciably more resources than estimated will be required for optimal management of cardiovascular risk factors and secondary prevention of cardiovascular disease (CVD).

The British women's heart and health study showed that among 4286 women prevalences of disease and risk factors were higher than supposed. A fifth had CVD and half high blood pressure, 12% smoked, 27% were obese, and 21% were inactive. Half had a total cholesterol concentration > 6.5 mmol/l. Age adjusted odds of CVD was highest in Scotland and lowest in south England. The odds narrowed, but were not equivalent, after adjusting further for age, risk factors, socioeconomic class, and treatment.

Control of risk factors and secondary preventive measures in women with CVD seemed as governed by region, socioeconomic class, and age as by clinical need but was suboptimal everywhere, according to the prevalences of smoking (12%), uncontrolled blood pressure (33%), obesity (30%), and total cholesterol concentration > 5 mmol/l (90%) or aspirin or antiplatelet treatment (41%) or statin treatment (22%).

The study was modelled on the British regional heart study, and women aged 60–79 years were sampled similarly across 23 towns in Scotland, northern England, Midlands and Wales, and south England. Data were obtained from GP records, self completion and nurse administered questionnaires, and physical examination and testing.

The distribution of risk factors for CVD and their control in women aged 60 plus was not known accurately. This study set out to remedy this in a nationally representative sample.

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