Haemostatic and haemodynamic abnormalities associated with left atrial thrombosis in non-rheumatic atrial fibrillation

R M Heppell, K E Berkin, J M McLenachan, J A Davies

Abstract

Objectives—to evaluate the role of haemostatic and haemodynamic variables in left atrial thrombosis in non-rheumatic atrial fibrillation.

Design—Case-control study.

Subjects—one hundred and nine patients with non-rheumatic atrial fibrillation.

Interventions—Peak blood velocity measured at three sites in the left atrium. Venous blood sampled for coagulant proteins and markers of haemostatic activation.

Main outcome measures—Presence of left atrial thrombus and spontaneous echo contrast at transoesophageal echocardiography.

Results—Left atrial thrombus was identified in 19 patients (18%), 16 of whom had spontaneous echo contrast. Patients with thrombus had reduced peak left atrial appendage velocity compared with those without (0·17 v 0·26 m/s; P < 0·001), but no significant reductions in peak mid-left atrial or mitral valve outflow velocity. Patients with thrombus had increased plasma markers of platelet activation—β thromboglobulin (56·8 v 30·4 IU/ml; P < 0·001) and platelet factor 4 (6·1 v 3·5 IU/ml; P < 0·01)—and of thrombogenesis: thrombin-antithrombin complexes (5·59 v 3·06 μg/ml; P < 0·001) and D-dimers (479 v 298 ng/ml; P < 0·01). von Willebrand factor was also increased (1·81 v 1·52 IU/ml; P < 0·05). A multiple logistic regression model identified left atrial appendage velocity (P = 0·001), β thromboglobulin (P = 0·002), and von Willebrand factor (P = 0·04) as the independent associates of left atrial thrombosis, ahead of the presence of spontaneous echo contrast.

Conclusions—Haemostatic and haemodynamic abnormalities are associated with left atrial thrombus in non-rheumatic atrial fibrillation, and may help stratify thromboembolic risk.

Methods

SUBJECTS
Between October 1992 and December 1994, 109 subjects with non-rheumatic atrial fibrillation were recruited from The General Infirmary and St James’s University Hospital, Leeds. The study was approved by the research
ethics committee of the United Leeds Teaching Hospitals Trust. Cases with evidence of atrial fibrillation on their presenting ECG were recruited consecutively from the inpatient and outpatient departments. The arrhythmia was confirmed at the time of venous blood sampling and at echocardiography. Patients with evidence of sinus rhythm on either of these occasions were documented as having paroxysmal atrial fibrillation (PAF). Subjects were excluded if they had evidence of rheumatic heart disease (history of rheumatic fever, clinical or echocardiographic evidence of mitral stenosis) or had a prosthetic heart valve.

Current or recent (less than four weeks) oral anticoagulant or heparin usage, or the presence of underlying conditions likely to affect haemostasis (recent surgery, active infection, inflammation, or malignancy) were exclusion criteria, as was a recent (less than four weeks) thrombotic event, including venous thromboembolism, myocardial infarction, or stroke.

Decisions about subsequent antithrombotic treatment were left to the patients’ attending physician.

VENOUS BLOOD SAMPLING

Samples were taken between 0900 and 1000 after 15 minutes of rest in the semirecumbent position. Venous blood was sampled from the antecubital fossa with a 19G butterfly needle, minimal stasis, and a multiple syringe technique. After discarding the first 5 ml, 9 ml of blood were sampled into 1 ml of 0·1 M sodium citrate at room temperature, 9 ml into 1 ml of cold citrate (4°C), 4·5 ml into 0·5 ml of heparin/protinin anticoagulant (4°C) for fibrinopeptide A estimation, and 4·5 ml into a glass tube containing 0·5 ml of a citrate, adenosine, dipyrindaline, and theophylline solution (Diatube H, 4°C) (Diagnostica Stago, France) for markers of platelet activation. Within 20 minutes of venepuncture, room temperature samples were centrifuged at 2000 × g for 20 minutes and cold samples at 3000 × g for 30 minutes at 4°C. The platelet-poor plasma was then snap frozen and stored at −40°C until assayed.

BIOLGICAL ASSAYS

The activity of coagulant proteins factor VII (coefficient of variation (CV) = 6%) and factor VIII (CV = 7·0%) was estimated using the one stage clotting time based assay, and plasma fibrinogen concentration was estimated by the method of Clauss (CV = 3·5%, normal range in our laboratory (NR) = 1·37–3·40 g/l). Von Willebrand factor antigen was quantified by enzyme immunoassay (EIA) (Dako, Copenhagen) according to the supplied manufacturer’s instructions (CV = 4·7%, NR = 0·86–1·18 IU/ml). Markers of platelet activation, β thromboglobulin (CV = 8·0%, NR = 10–40 IU/ml), and platelet factor 4 (PF4, CV = 12%, NR = 0·5–5·0 IU/ml) were quantified by EIA using the manufacturer’s instructions (Diagnostica Stago). D-dimer concentrations (CV = 6·7%, NR < 400 ng/ml, Diagnostica Stago), and markers of haemostatic activation: thrombin-antithrombin complexes (TAT, CV = 13%, NR = 1·0–4·1 μg/l, Behringwerke, Marburg), prothrombin fragment 1+2 (F 1+2, CV = 8·6%, NR = 0·44–1·1 nmol/l, Behringwerke), and fibrinopeptide A (FPA, CV = 12%, NR < 3 ng/ml, Diagnostica Stago) were assayed using EIA according to instructions supplied.

TRANSOEosophageal Echocardiography

Transoesophageal echocardiography was attempted in 109 subjects using a Toshiba 5 MHz single plane probe or a Vingmed 5 MHz multiplane probe. Images were analyzed on-line by two observers (RMH and either KEB or JMM) for the presence of left atrial thrombus and spontaneous echo contrast. Thrombus was defined as a discrete echodensity, visible in more than one plane, of > 5 mm diameter and acoustically distinct from underlying endocardium (fig 1). Spontaneous echo contrast was defined as a swirling non-homogeneous echo seen in the left atrium and distinguishable from background tissue or speckle by manipulation of gain settings (fig 2). Differences between observers were resolved by consensus; if observers could not agree, the feature was deemed not to be present.

Pulsed wave Doppler ultrasound was used to estimate peak blood velocity at three sites within the left atrium: the mouth of the left atrial appendage directed towards the probe, the mid-left atrium (mid-LA) toward the mitral valve orifice, and a point 5 mm on the left atrial side of the mitral leaflet co-apt point directed through the valve (MV outflow). For each site, pulsed wave Doppler spectra were recorded on videotape for off-line analysis. Peak velocities were measured for 5–10 cardiac cycles at each site, and averaged to overcome beat to beat variation.

ANALYSIS

Univariate analyses were performed between patients with and without thrombus, using unpaired t tests on haemodynamic variables, Mann-Whitney tests for haemostatic variables, and χ² tests with Yates’ correction for categorical variables. A multiple logistic regression

Figure 1 Thrombus seen in the left atrial appendage.
Haemostatic and haemodynamic abnormalities associated with left atrial thrombosis in non-rheumatic atrial fibrillation

Model was constructed using a stepwise technique, including all variables with univariate P < 0·1. Analyses were performed using SPSS 6·1 for Windows.

Table 1 Clinical characteristics of study patients

<table>
<thead>
<tr>
<th></th>
<th>Left atrial thrombus</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>19</td>
<td>88</td>
</tr>
<tr>
<td>Women (%)</td>
<td>6 (32)</td>
<td>32 (36)</td>
</tr>
<tr>
<td>Men (%)</td>
<td>13 (68)</td>
<td>56 (64)</td>
</tr>
<tr>
<td>Mean (SD) age (years)</td>
<td>73·0 (10·4)</td>
<td>68·6 (9·1)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>9 (47)</td>
<td>36 (43)</td>
</tr>
<tr>
<td>Ischaemic heart disease (%)</td>
<td>9 (47)</td>
<td>31 (35)</td>
</tr>
<tr>
<td>PAF (%)</td>
<td>1 (5·3)</td>
<td>13 (15)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>3 (16)</td>
<td>16 (18)</td>
</tr>
<tr>
<td>Previous CVA (%)</td>
<td>3 (16)</td>
<td>20 (23)</td>
</tr>
<tr>
<td>Aspirin use (%)</td>
<td>10 (53)</td>
<td>44 (50)</td>
</tr>
</tbody>
</table>

PAF, paroxysmal atrial fibrillation; CVA, cerebrovascular accident.

Table 2 Mean peak velocity (SD) at three sites in the left atrium

<table>
<thead>
<tr>
<th></th>
<th>Left atrial thrombus</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAA outflow (m/s)</td>
<td>0·17 (0·07)*</td>
<td>0·26 (0·12)</td>
</tr>
<tr>
<td>Mid-LA (ms)</td>
<td>0·23 (0·10)</td>
<td>0·28 (0·11)</td>
</tr>
<tr>
<td>MV outflow (m/s)</td>
<td>0·39 (0·12)</td>
<td>0·46 (0·14)</td>
</tr>
</tbody>
</table>

LAA, left atrial appendage; LA, left atrium; MV, mitral valve.

*P < 0·001 v absent group.

Table 3 Median (interquartile range) values for haemostatic variables studied

<table>
<thead>
<tr>
<th></th>
<th>Left atrial thrombus</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet activation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β thromboglobulin (IU/ml)</td>
<td>56·8 (44·1 to 77·6)‡</td>
<td>30·4 (24·5 to 43·9)</td>
</tr>
<tr>
<td>Platelet factor 4 (IU/ml)</td>
<td>6·1 (3·6 to 11·4)‡</td>
<td>3·5 (2·0 to 6·4)</td>
</tr>
<tr>
<td>Haemostatic activation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombin-antithrombin complex (μg/ml)</td>
<td>5·59 (3·90 to 6·93)‡</td>
<td>3·06 (2·06 to 4·81)</td>
</tr>
<tr>
<td>Prothrombin fragment 1 + 2 (nM/ml)</td>
<td>1·55 (1·19 to 2·46)</td>
<td>1·53 (1·09 to 1·87)</td>
</tr>
<tr>
<td>Fibrinopeptide A (ng/ml)</td>
<td>3·14 (2·22 to 8·22)</td>
<td>2·44 (1·56 to 4·04)</td>
</tr>
<tr>
<td>Coagulant proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>von Willebrand factor (IU/ml)</td>
<td>1·81 (1·4 to 2·8) *</td>
<td>1·52 (1·18 to 2·08)</td>
</tr>
<tr>
<td>Factor VII:C (IU/ml)</td>
<td>0·91 (0·73 to 1·09)</td>
<td>0·91 (0·80 to 1·17)</td>
</tr>
<tr>
<td>Factor VIII:C (IU/ml)</td>
<td>2·05 (1·64 to 2·64)</td>
<td>1·96 (1·51 to 2·34)</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>3·64 (3·05 to 4·55)</td>
<td>3·40 (2·82 to 4·24)</td>
</tr>
</tbody>
</table>

D-dimer (ng/ml) | 479 (334 to 738)† | 298 (175 to 502) |

‡P < 0·001, †P < 0·01, *P < 0·05 v absent group.

Results

The left atrium and left atrial appendage were visualised adequately in 107 of 109 patients. In one subject the probe could not be advanced because of an unrecognised pharyngeal pouch; in one other, inadequate views of the left atrial appendage were obtained. Left atrial thrombus was identified in 19 patients (18%). Eighteen patients had thrombus in the left atrial appendage only; one had thrombus in the left atrial appendage and in the body of the left atrium.

Spontaneous echo contrast was present in some part of the left atrium in 67 (63%) patients; of the 19 with left atrial thrombus, 16 had spontaneous echo contrast (84%) (χ² = 3·55, P = 0·06).

Clinical characteristics of patients with and without left atrial thrombus are shown in table 1. There were no significant differences between the groups, although patients with thrombus tended to be older than those without (73 (10) v 69 (9) years; P = 0·07).

Table 2 shows the mean (SD) peak velocities at three sites in the left atrium for patients with and without thrombus. Peak left atrial appendage outflow velocity was significantly lower in the thrombus group (0·17 (0·07) m/s v 0·26 (0·12) m/s; P < 0·0001).

Table 3 shows the median values and interquartile ranges for the haemostatic variables measured. Markers of platelet activation, β thromboglobulin (P < 0·0001) and platelet factor 4 (P = 0·007) were raised in the thrombus group, as were thrombin–antithrombin complexes (P = 0·0007) and D-dimers (P = 0·004), indicating activation of coagulation and accelerated fibrin turnover. von Willebrand factor antigen titres were also higher in the thrombus group (P = 0·02), without a corresponding increase in factor VIII:C. Plasma fibrinogen concentrations did not differ significantly between the two groups.

With the exception of an overall reduction in β thromboglobulin concentrations (31 v 37·2 IU/ml; P = 0·04), aspirin use was not associated with significant differences in the haemostatic variables studied. Figure 3 shows significantly higher β thromboglobulin concentration in thrombus patients, whether taking aspirin or not.

Figure 2 Spontaneous echo contrast in the left atrium. AV, atrial valve; MV, mitral valve.

Figure 3 Boxplot of median (interquartile range) β thromboglobulin concentrations in patients with and without left atrial (LA) thrombus, according to aspirin use.
Table 4  Independent correlates of left atrial thrombus

<table>
<thead>
<tr>
<th>Variable</th>
<th>R*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak LAA velocity</td>
<td>-0.25</td>
<td>0.001</td>
</tr>
<tr>
<td>β thromboglobulin</td>
<td>0.23</td>
<td>0.002</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>0.13</td>
<td>0.04</td>
</tr>
<tr>
<td>Age</td>
<td>0</td>
<td>n.s.</td>
</tr>
<tr>
<td>SEC</td>
<td>0</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

LAA, left atrial appendage; SEC, spontaneous echo contrast. *Partial correlation coefficient.

A multiple logistic regression model was developed to define the variables most strongly associated with the presence of left atrial thrombus, including age as a possible confounding variable. Table 4 shows the partial correlation coefficients and significance levels for the variables most strongly identified with left atrial thrombus. Reduced left atrial appendage velocity, raised β thromboglobulin, and raised von Willebrand factor antigen were independent correlates of left atrial thrombus; there was no independent relation with age or spontaneous echo contrast.

Discussion
The value of haemostatic and haemodynamic abnormalities in predicting future stroke is not directly addressed by this case-control study. Subjects without visible thrombus may have had previous thromboembolism, or be at risk of future events. We can, however, be reasonably sure that those in the left atrial thrombus group did have left atrial thrombosis. Transoesophageal echocardiography can detect left atrial thrombus with a high degree of accuracy,15,16 with recently reported sensitivity and specificity of 100% and 99%, respectively using a two observer technique compared with direct inspection at operation.17 Our thrombus detection rate of 18% is in line with other series of cases of non-rheumatic atrial fibrillation.18-20 The initial use of a single plane probe may have reduced the sensitivity for detection of left atrial thrombus; however, all thrombi detected using the multiplane probe were visible in single plane mode.

There were no differences in the prevalence of previous stroke, ischaemic heart disease, hypertension, or smoking between patients with thrombi and those without; it is therefore unlikely that these account for the haemostatic differences observed. Likewise, use of aspirin was distributed evenly between the groups; this affected overall concentrations of β thromboglobulin alone, but not the differences noted between patients with and without thrombi. While patients with paroxysmal atrial fibrillation may show less evidence of haemostatic activation than their chronic counterparts,21 the prevalence of this arrhythmia was not significantly different between the two groups. Patients with thrombus tended to be older than those without; age, however, was not independently associated with left atrial thrombus.

Virchow identified a triad of components implicated in the process of thrombosis:22 abnormal conditions of blood flow, vessel wall damage, and abnormal blood constituents. In this study we examined variables relating to all three components of the triad. The loss of atrial systole in atrial fibrillation, and the increased relative risk of stroke associated with rheumatic atrial fibrillation point strongly towards a role for stasis of blood in left atrial thrombosis. Previous studies have associated abnormal blood flow in the left atrial appendage with atrial fibrillation,23,24 particularly in patients with mitral stenosis,25 or with a history of presumed cardioembolism.26 Left atrial thrombus has been correlated with reduced left atrial appendage velocities,23,25 increased left atrial and left atrial appendage size,24,26 and the presence of spontaneous echo contrast.3,4,14 Our data support the correlation of reduced left atrial appendage velocity with left atrial thrombus, and suggest that this measure is a more powerful risk marker for thrombosis than spontaneous echo contrast. Spontaneous echo contrast has been associated with reduction in velocities elsewhere in the left atrium27; we suggest that some measure of left atrial appendage blood flow is more likely to contribute to the risk of predominantly left atrial appendage thrombosis in non-rheumatic atrial fibrillation.

Independent of the reductions in blood velocity, we found evidence suggesting that endothelial dysfunction and haemostatic activation are associated with left atrial thrombosis. von Willebrand factor is released from damaged endothelium, and has a role in platelet aggregation through a receptor on the platelet surface. Previous studies have shown raised plasma von Willebrand factor in subjects with atrial fibrillation compared to controls in sinus rhythm,10,11 with a suggestion of higher levels in patients with enlarged left atria. von Willebrand factor is released during platelet aggregation, and its concentration in the peripheral circulation is increased in a variety of conditions associated with vascular injury; it may therefore be a reflection of platelet activation occurring in left atrial thrombosis, or a marker of local (endocardial) or widespread endothelial damage.

We found further evidence that platelet activation, with raised concentrations of platelet factor 4, and in particular β thromboglobulin, was associated with left atrial thrombus. Concentrations of both these variables are raised in subjects with atrial fibrillation compared to controls in sinus rhythm;2 two previous studies showed raised concentrations of β thromboglobulin in ischaemic stroke patients.28,29

In general, other studies in patients with atrial fibrillation have shown similar coagulation abnormalities to those observed in ours when compared with controls in sinus rhythm. With the exception of fibrinogen, we have found that such abnormalities are greatest in the group of non-rheumatic patients with atrial fibrillation who are shown to have left atrial thrombus. We found no differences in plasma fibrinogen: concentrations in the two groups were comparable to those in the atrial fibrillation group of Lip et al.11 We did, however, find evidence of increased thrombin gen-
\section*{Haemostatic and haemodynamic abnormalities associated with left atrial thrombosis in non-rheumatic atrial fibrillation}

Haemostatic abnormalities have been found in a wide variety of thrombotic disorders, including stroke, ischaemic heart disease, 

Haemostatic abnormalities and left atrial thrombosis is unclear; increases in markers of platelet and haemostatic activation suggest a reflection of thrombosis rather than a causal role. These circulating haemostatic markers may have a role in generating further thrombosis. Whether these markers are generated in the left atrium is not known, and although one study during balloon valvoplasty has indicated abnormalities of haemostasis in blood sampled from the left atrium, it may be that peripheral sampling underestimates the degree of haemostatic activation in left atrial thrombosis because of haemodilution.

Determinants of left atrial thrombosis, and better understanding of its formation, should be beneficial in stratifying risk of stroke for the individual with atrial fibrillation. This study indicates that, in particular, left atrial appendage peak velocity and markers of platelet or haemostatic activation show promise as indicators of thromboembolic risk in non-rheumatic atrial fibrillation.

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