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Activation of the endogenous coagulation system in patients with atrial flutter: Relationship to echocardiographic markers of thromboembolic risk

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Abstract

Background: *Atrial thrombus formation in patients with atrial flutter raises concerns of stroke risk. We investigated patients with isthmus-dependent atrial flutter for coagulation abnormalities before and after cardioversion to sinus rhythm by catheter ablation, and evaluated the relationship of the abnormalities to the echocardiographic risk markers of stroke.*

Methods and results: *Plasma samples were drawn prior to insertion of catheters, immediately after the procedure, and 24 hours afterwards. At baseline, coagulation abnormalities were found in 22 out of 25 patients (88%). von Willebrand factor antigen (vWF-Ag) and factor VIII:C were elevated in 17 patients (68%) and 15 patients (60%), respectively. At baseline, mean plasma levels of vWF-Ag (250.1 ± 144.4%) and factor VIII:C (215.0 ± 77.1%) were increased. Key markers of thrombin generation, thrombin-antithrombin III complex (TAT; 47.8 ± 30.9 μg/L vs 14.5 ± 13.8 μg/L; $p < 0.05$) and prothrombin fragments 1.2 (F1.2; 2.5 ± 0.5 nmoL/L vs 1.2 ± 1.0 nmoL/L) were significantly elevated in the presence of spontaneous echo contrast. Further, both markers of thrombin generation inversely correlated with left atrial appendage emptying velocity ($r = –0.42$ and $–0.63$, $p < 0.05$). Levels of TAT and F1.2 increased after conversion and ablation.*

Conclusions: *Endothelial-dependent coagulation factors were enhanced in most patients with atrial flutter. Spontaneous echo contrast and decreased atrial contractility were associated with increased thrombin generation. After conversion and ablation, an increase in thrombin generation and fibrinolysis suggest a transient pro-thrombotic state.* (Cardiol J 2010; 17, 4: 390–396)

Key words: atrial flutter, coagulation, thromboembolic risk, stroke, ablation

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Introduction

Reports of atrial thrombus on transesophageal echocardiography and embolic events after cardioversion of atrial flutter (AFL) suggest a greater risk of stroke than previously thought [1–4]. After termination of AFL by direct current cardioversion or radiofrequency (RF) ablation, patients have an increased incidence of thromboembolism and frequently demonstrate spontaneous echo contrast and decreased left atrial appendage emptying velocity [3, 5–9]. In this study, we investigated the potential abnormalities of the coagulation system associated with AFL before and after conversion by ablation. Further, given the paucity of published data, we studied the relationship of coagulation abnormalities to the echocardiographic markers of thromboembolic risk.

Methods

Patient selection and study design

The study group consisted of 25 consecutive patients with drug-refractory AFL undergoing RF ablation. The 12-lead electrocardiogram showed f-wave morphology consistent with typical counterclockwise or clockwise AFL. Isthmus-dependent flutter was confirmed by right atrial mapping and entrainment at sub-eustachian isthmus. After confirmation, RF ablation was performed in the standard fashion. Warfarin was held for > five days prior to procedure in six patients; 19 patients had not been anticoagulated. Aspirin was not held prior to the procedure and none of the included patients were on clopidogrel.

The control group included ten patients who underwent ablation for other supraventricular tachycardias. Excluded from this study were patients with coexistent paroxysmal atrial fibrillation (AF), anticoagulation with heparin or warfarin during the five days preceding the ablation procedure, prosthetic heart valve, recent thrombotic or thromboembolic event (< four weeks), conditions likely to affect hemostasis or activation of the clotting system (e.g. recent surgery, infection, inflammation, malignancy, etc.), and patients unable or unwilling to consent to transesophageal echocardiography. The study was approved by the local Institutional Review Board and all patients provided signed informed consent.

Blood sampling and measurement of coagulation parameters

Venous blood was drawn before catheter insertion and immediately after the procedure in both study groups, and another sample was drawn 24 hours post-ablation in the AFL group. Blood was drawn from the antecubital vein to avoiding clotting or extravasation. Blood was mixed with sodium citrate solution (ratio 9:1), and centrifuged at 1500 xg for at least 10 min. Supernatant plasma was withdrawn and stored in several aliquots at –80°C until assay, and thawed at 37°C immediately prior to analysis. As markers of endothelium dependent coagulation, we measured von Willebrand factor antigen (vWF--Ag; normal range 54–163%) and factor VIII:C (normal range 69–182%). As markers of thrombin generation, we measured prothrombin fragments 1.2 (F1.2; normal range 0.4–1.1 nmoL/L) and thrombin-antithrombin III complex (TAT III; normal range 1.0–4.1 μg/L). And to assess fibrinolytic activity, we measured fibrinogen (normal range 200–400 mg/dL) and D-dimer levels (normal range < 0.44 μg/mL).

All assays were performed in batches according to reagent manufacturer recommendations. vWF-Ag activity was measured by enzyme-linked immunoabsorbent assay (ELISA). Activity of factor VIII:C was assessed by a standard one-stage clotting time. The fibrinogen activity was determined by the Clauss clotting technique and D-dimer levels by an immunoturbidimetric method. All of the above measurements were performed using reagents and instrumentation (Diagnostica Stago, Asnieres-sur-Seine, France). F1.2 and TAT were measured by ELISA using commercially available kits (Behringwerke, Marburg, Germany). All microtitreplate measurements were performed with a Dynatech MR4000 (Dynex Laboratories, Chantilly, VA, USA).

Echocardiographic studies and analysis

All patients underwent transthoracic and transesophageal echocardiography (TEE) before the invasive procedure. Measured TEE parameters included left ventricular ejection fraction (LVEF) and dimensions, left atrial diameter, left atrial appendage (LAA) function, and presence of thrombus and/or spontaneous echo contrast (SEC; defined as dynamic smoke-like echocardiographic images with a swirling motion that persisted after decreasing the gain). Left atrial appendage emptying velocity (LAA-EV) was assessed by pulsed wave Doppler placing the sample volume 1.5 cm into the mouth of the atrial appendage in the basal transverse plane at the level of the aortic valve. Peak flow velocities at end-diastole were measured and averaged over six cardiac cycles. Measured transthoracic echo parameters included right and left atrial dimensions (right atrial enlargement was defined

Table 1. Clinical characteristics (n = 25).

Age (years)	60 ± 8
Male	17 (68%)
Median duration of AFL (days)	222
Rate of AFL (beats/min)	255 ± 27
Ventricular rate in AFL (beats/min)	85 ± 18
Cardiovascular disease:	17 (68%)
Hypertension	17 (68%)
Diabetes mellitus	3 (12%)
Coronary artery disease	10 (40%)
Cardiomyopathy	7 (28%)

AFL — atrial flutter

Table 2. Echocardiographic findings (n = 25).

Left ventricular ejection fraction	40 ± 16%
Left ventricular ejection fraction < 35%	11 (44%)
Right ventricular dysfunction:	
Mild/moderate	8 (32%)
Severe	5 (20%)
Pulmonary hypertension:	
Mild (PAS > 35 mm Hg)	7 (28%)
Moderate (PAS > 50 mm Hg)	1 (4%)
Severe (PAS > 65 mm Hg)	1 (4%)
Mitral regurgitation:	
Mild	11 (44%)
Moderate	3 (12%)
Severe	0
Right atrial enlargement	21 (84%)
Left atrial diameter [mm]	5.2 ± 0.3
Left atrial appendage emptying velocity [cm/s]	43 ± 19
Left atrial appendage emptying velocity ≤ 30 cm/s	10 (40%)
Spontaneous echo contrast	4 (16%)

PAS — pulmonary artery systolic pressure

as right atrial area > 20 cm^2 in the four chamber view, left atrial diameters were measured in the para-sternal long axis view), LVEF (Simpson's method), pulmonary arterial pressure and assessment of valve function.

Statistical analysis

All continuous variables are expressed as mean ± standard deviation. Categorical variables are summarized as absolute number and relative frequencies (%). Changes in the pre and post measurements were compared using the non-parametric Wilcoxon matched-pairs signed ranks test. The Mann Whitney test was used to compare the change between the two groups. Analysis of variance for repeated measures was used to analyze changes in the pre-post and 24 hours measurements. Logistic regression analysis was used to identify correlates of elevation in coagulation parameters. SPSS version 15.0 (SPSS Inc, Chicago, IL) was used for the statistical analysis. Results with $p < 0.05$ were considered statistically significant.

Results

Clinical characteristics

Clinical data and echocardiographic findings (n = 25) are displayed in Tables 1 and 2. All 25 patients were successfully ablated.

Coagulation abnormalities

Abnormalities among the parameters tested were found in 22 of the 25 patients (88%). Both vWF-Ag and factor VIII:C were frequently elevated [17 patients (68%) and 15 patients (60%), respectively] and mean plasma levels of vWF-Ag and factor VIII:C were increased (Fig. 1A) above normal values. Also, elevated levels of F1.2, TAT and fibrinogen were frequently encountered (Figs. 1B, C).

Multivariate analyses using baseline clinical parameters (age, gender, hypertension, coronary artery disease) identified the presence of coronary artery disease (HR = 33.5, CI 1.5–757.8, p = 0.03) to be correlated with higher fibrinogen levels. No other clinical parameters were correlated with other coagulation abnormalities.

Evolution of coagulation abnormalities after conversion and ablation

Both F1.2 ($p = 0.003$) and TAT levels ($p = 0.03$) were increased immediately following conversion and ablation, with a decrease 24 hours after the procedure (Fig. 2). No significant changes in levels of vWF-Ag were noted after conversion and ablation. There was a trend for D-dimer levels ($p = 0.08$) and factor VIII:C levels ($p = 0.08$) to be elevated after ablation compared to pre-ablation values and remain elevated 24 hours after ablation.

Relationship of coagulation abnormalities to echocardiographic parameters

At baseline, SEC was demonstrated in four of the 25 patients (16%) with AFL. Patients with SEC had significantly elevated markers of thrombin generation, F1.2 and TAT levels, when compared to patients without SEC ($p < 0.05$, Table 3). Plasma levels of F1.2 ($r = –0.63$, $p < 0.01$) and TAT ($r = –0.42$, $p < 0.05$) were inversely related to LAA-EV (Fig. 3, Table 4). Coagulation parameters did not

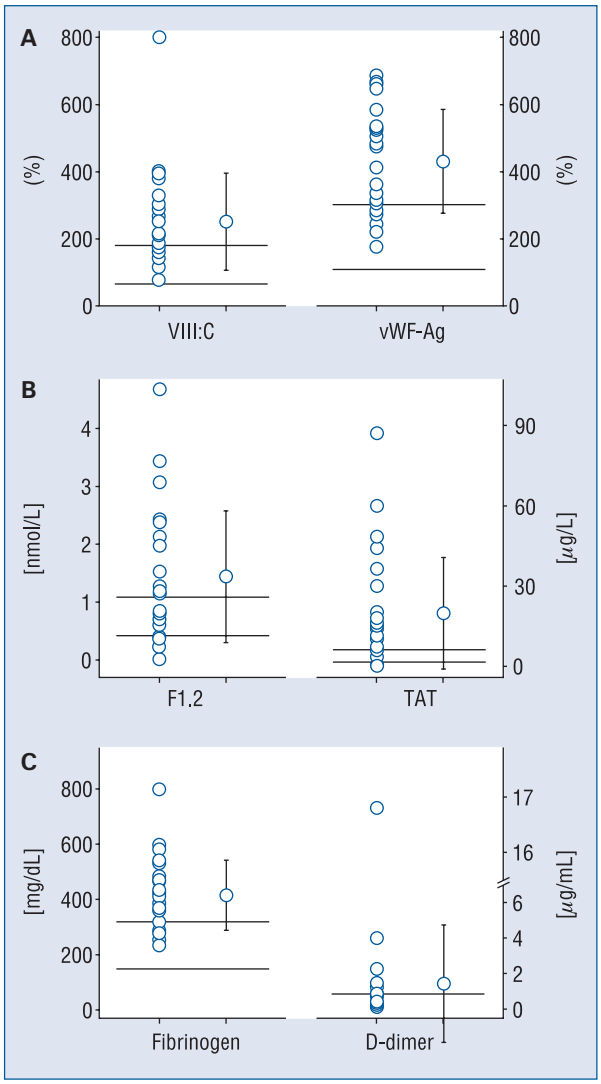

Figure 1. Baseline coagulation parameters: **A.** Endothelium dependent coagulation — factor VIII:C (left) and von Willebrand factor antigen — vWF-Ag (right); **B.** Thrombin generation: F1.2 (left) and TAT (right); **C.** Fibrinolytic activity: Fibrinogen (left) and D-dimer (right). Normal ranges indicated by the horizontal lines.

differ in patients with or without left ventricular dysfunction (Table 5).

Control group

At baseline, all coagulation parameters were within normal range, except a mild elevation in the TAT (7.4 ± 6.7 μg/L) was noted. Immediately post-ablation, there was a significant increase in the elevated markers of thrombin generation, F1.2 and TAT levels, but these values still remained well below baseline values in the AFL group (Table 6).

Discussion

This study demonstrates activation of the coagulation system in patients with isthmus-dependent AFL. Patients with echocardiographic evidence of spontaneous echo contrast and decreased LAA-EV had elevated levels of thrombin generation. Evidence of increased thrombin generation (elevated F1.2 and TAT) and intravascular fibrin turnover (decreased fibrinogen and increased D-dimer levels) after conversion and ablation raise the possibility of a transient pro-thrombotic state following ablation and/or termination of AFL. Patients with supraventricular tachycardia did not show evidence of coagulation system activation at baseline and had lesser activation following the ablation procedure.

Implications of endothelial-dependent activation of the coagulation system

In most patients, vWF-Ag and factor VIII:C levels were elevated at baseline. These results accord with data reported in patients with non-valvular AF [10, 11]. Plasma levels of vWF-Ag and factor VIII:C did not correlate with LAA-EV, LVEF or the presence or absence of SEC. vWF-Ag is important in mediating platelet adhesion and is a marker for endothelial dysfunction. Immunohistochemistry studies of vWF-Ag in endothelial cells harvested from the LAA of patients with and without AF undergoing heart surgery, or at autopsy in normal controls, revealed an increase of immunoreactive vWF-Ag in the endocardium of the pressure overloaded atrial appendage (particularly with mitral valve disease), irrespective of AF [12]. The finding that elevated plasma levels of vWF-Ag and factor VIII:C in most of our patients with AFL may reflect a coagulation abnormality at the endocardial level which is independent of stasis or poor atrial contractile function. In this study, coronary artery disease was associated with higher fibrinogen levels. These results are similar to previous studies that demonstrated markers of inflammation (fibrinogen, C-reactive protein and serum amyloid) are positively associated with prevailing coronary artery disease [13].

Evidence of increased thrombin generation and fibrinolytic activity after isthmus ablation

Markers of thrombin generation (F1.2 and TAT) levels increased one hour after conversion and ablation and remained elevated above pre-ablation levels even 24 hours after ablation (Fig. 3).

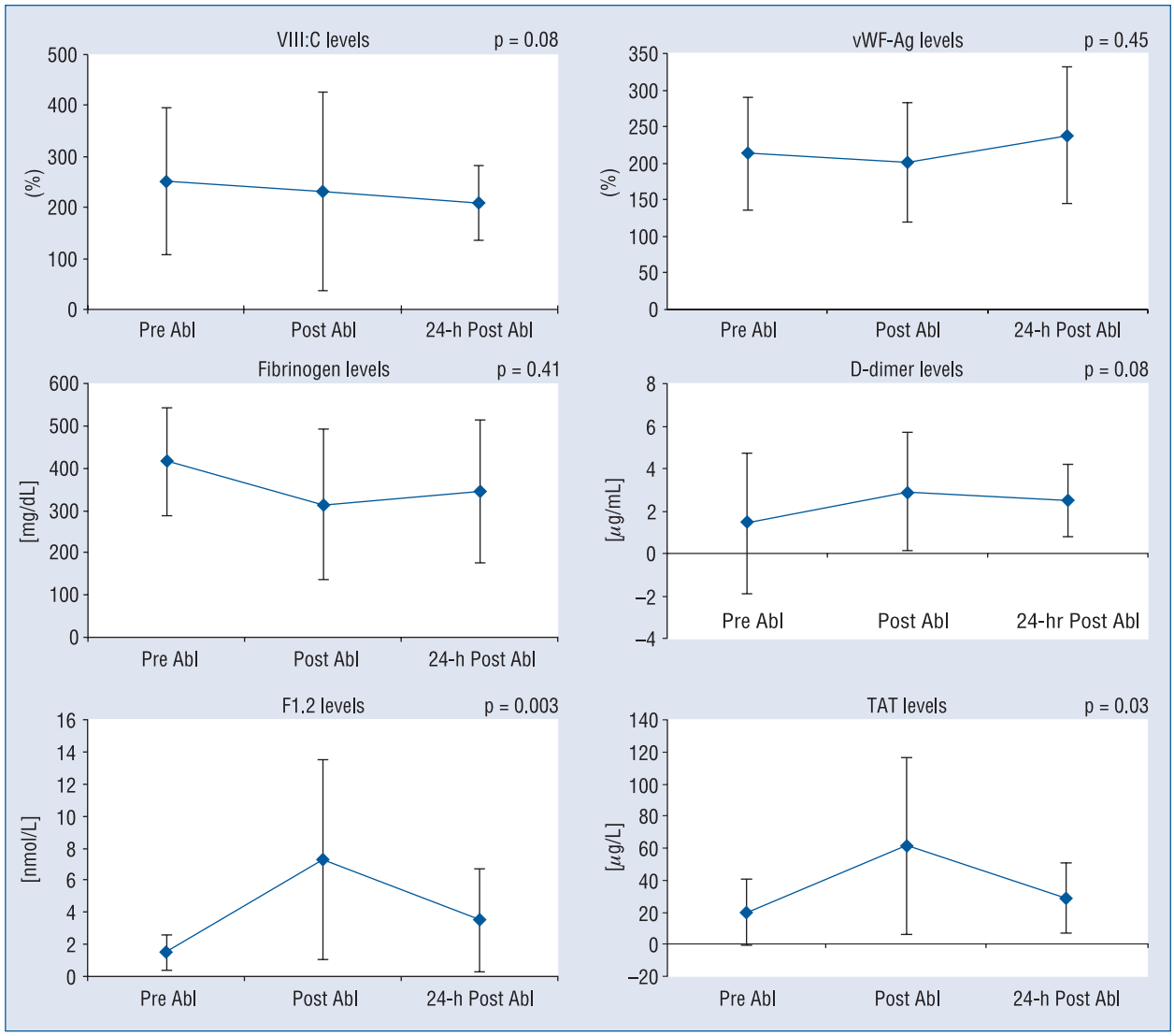

Figure 2. Coagulation parameters before and after conversion and ablation. An increased level of F1.2 and TAT immediately after ablation (bottom row) is consistent with enhanced thrombin generation during the first hours following ablation. The trend towards elevated D-dimer levels (middle row) persisting beyond the first 24 hours after ablation suggests increased fibrinolytic activity; Abl — ablation.

Table 3. Relationship of presence of spontaneous echo contrast (SEC) and coagulation system activity.

	SEC (n = 4)	No SEC (n = 21)	P
vWF-Ag (%)	243 ± 86	210 ± 76	NS
Factor VIII:C (%)	269 ± 38	247 ± 157	NS
F1.2 [nmol/L]	2.5 ± 0.5	1.2 ± 1.0	< 0.05
TAT [μg/L]	47.8 ± 30.9	14.5 ± 13.8	< 0.01
Fibrinogen [mg/dL]	490 ± 95	401 ± 129	NS
D-dimer [μg/mL]	1.3 ± 0.9	1.5 ± 3.6	NS

vWF-Ag — von Willebrand factor antigen; TAT — thrombin-antithrombin III complex; F1.2 — prothrombin fragments 1.2

D-dimer levels increased after isthmus ablation, and fibrinogen levels tended to decrease consistent with increased fibrin formation and fibrinolytic activity following flutter ablation and/or termination. Our study is consistent with recent data suggesting that ablation of AFL induces a prothrombotic state as evidenced by elevation of procoagulant microparticles [14]. Similarly to our study, vWF and D-dimer levels remained elevated post-ablation reflecting an ongoing prothrombotic state [14]. Enhanced thrombin generation and intravascular fibrin turnover after ablation could be due to activation of coagulation system during venous sheath and catheter placement, endocardial injury after delivery of RF

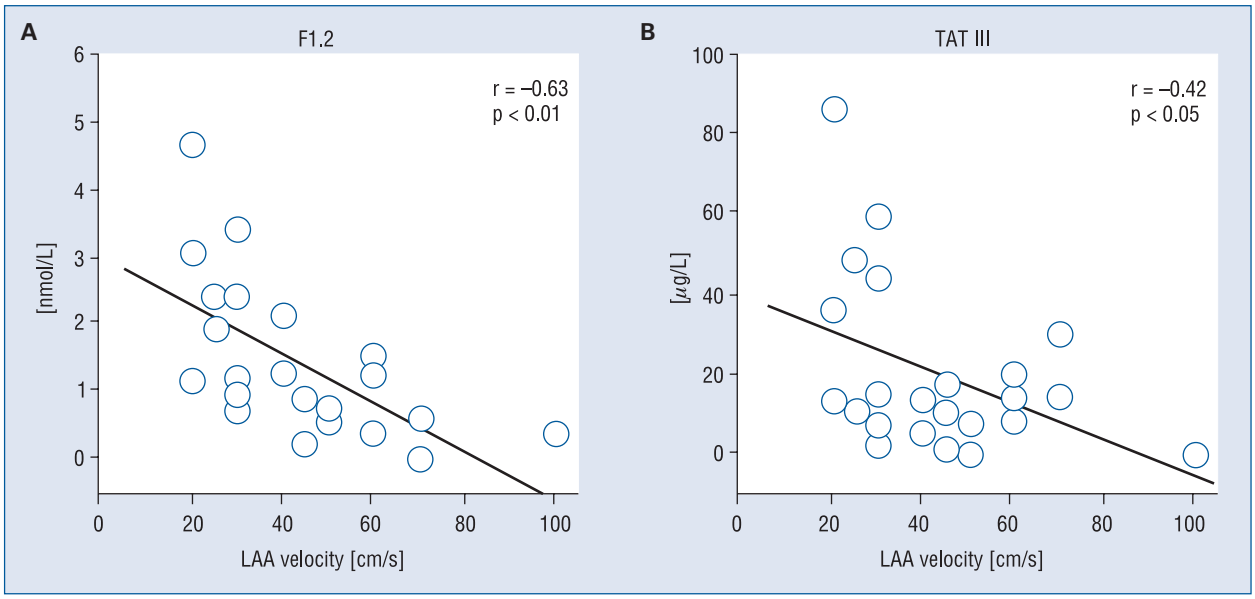

Figure 3. Correlation of left atrial appendage (LAA) emptying velocity with markers of thrombin generation. Plasma levels of F1.2 (**A**) and TAT III (**B**) were inversely related to left atrial appendage velocity.

Table 4. Relationship of left atrial appendage emptying velocity (LAA-EV) and coagulation system activity.

	LAA-EV ≤ 30 cm/s (n = 10)	LAA-EV > 30 cm/s (n = 15)	P
vWF-Ag (%)	232 ± 80	204 ± 76	NS
Factor VIII:C (%)	250 ± 76	252 ± 179	NS
F1.2 [nmol/L]	2.1 ± 1.2	1.0 ± 0.9	< 0.05
TAT [μg/L]	33.5 ± 26.6	10.7 ± 8.2	< 0.05
Fibrinogen [mg/dL]	470 ± 89	379 ± 138	0.08
D-dimer [μg/mL]	2.52 ± 5.06	0.70 ± 0.98	NS

vWF-Ag — von Willebrand factor antigen; TAT — thrombin-antithrombin III complex; F1.2 — prothrombin fragments 1.2

Table 5. Relationship of left ventricular ejection fraction (LVEF) and coagulation system activity.

	LVEF ≤ 35% (n = 11)	LVEF > 35% (n = 14)	P
vWF-Ag (%)	219 ± 91	212 ± 68	NS
Factor VIII:C (%)	298 ± 184	214 ± 95	NS
F1.2 [nmol/L]	1.7 ± 1.4	1.2 ± 0.9	NS
TAT [μg/L]	22.7 ± 24.9	17.6 ± 17.6	NS
Fibrinogen [mg/dL]	427 ± 107	406 ± 144	NS
D-dimer [μg/mL]	2.37 ± 4.92	0.69 ± 0.58	NS

vWF-Ag — von Willebrand factor antigen; TAT — thrombin-antithrombin III complex; F1.2 — prothrombin fragments 1.2

Table 6. Baseline and post-ablation coagulation parameters in the control group.

	Pre-ablation (n = 10)	Post-ablation (n = 10)	P
vWF-Ag (%)	86.2 ± 28.2	98.0 ± 37.6	NS
Factor VIII:C (%)	88.6 ± 62.2	82.1 ± 28.3	NS
F1.2 [nmol/L]	0.9 ± 0.6	1.4 ± 0.6	0.05
TAT [μg/L]	7.4 ± 6.7	15.6 ± 22.1	NS
Fibrinogen [mg/dL]	328.0 ± 72.8	344.5 ± 53.2	NS
D-dimer [μg/mL]	0.4 ± 0.2	1.8 ± 1.1	0.02

vWF-Ag — von Willebrand factor antigen; TAT — thrombin-antithrombin III complex; F1.2 — prothrombin fragments 1.2

energy, and/or atrial stunning after termination of AFL [14, 15]. This continuation of prothrombotic and increased fibrinolytic state post-ablation would necessitate continuation of anticoagulation following ablation of AFL.

Enhanced thrombin generation and impaired atrial contractility

Previous studies have demonstrated elevated plasma levels of fibrinogen and D-dimer in patients with chronic AF [10, 11, 13, 16]. In patients with AF, Black et al. [1] have reported that SEC was associated with increased plasma fibrinogen, suggesting a hypercoagulable state in addition to stasis.

This study revealed elevated plasma levels of TAT in patients with AFL. Similar studies in patients with AF have demonstrated that 1) TAT is elevated compared to age-matched control subjects [13], and 2) TAT tends to be higher in patients with AF > 12 hours duration compared to < 12 hours in duration [16]. In patients with AFL, Sakurai et al. [17] reported that impaired LAA function and SEC was associated with elevated D-dimer and beta-thromboglobulin levels, suggesting a potentially higher risk for thromboembolism. This study confirmed these findings and further demonstrated that presence of SEC is associated with elevated levels of F1.2 and TAT, which also showed a moderate inverse correlation to LAA-EV. In addition, plasma fibrinogen levels tended to be higher in AFL patients with decreased LAA-EV.

Limitations of the study

It is possible that our findings might be in part secondary to cardiovascular disease or to the interruption of anticoagulation, although like AF, AFL is well documented to generate a thrombogenic state. However, normal baseline values of nearly all coagulation parameters in the control group suggest that activation of coagulation system may indeed be related to AFL.

Conclusions

Patients with isthmus-dependent AFL demonstrated endothelial-dependent activation of the coagulation system and enhanced thrombin generation in the setting of decreased atrial contractility and SEC. A greater increase in thrombin and fibrinolytic activity was found after AFL termination by ablation. It remains to be determined how valuable these coagulation factors are as predictors of embolic events and stroke in patients with AFL.

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