The endothelium and atrial fibrillation
The prothrombotic state revisited

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Summary
Atrial fibrillation (AF) is the commonest sustained cardiac rhythm disorder which is associated with a high risk of mortality and morbidity from stroke and thromboembolism. The precise mechanisms by which AF causes thromboembolism and subsequent cerebrovascular events have attracted much research interest, and are yet to be fully elucidated. Nonetheless, it is well recognised that AF fulfils Virchow's triad for thrombogenesis, with abnormal flow conditions with loss of atrial contractility and an irregularly irregular cardiac output, (i.e. flow abnormalities), as well as structural heart disease with endocardial damage (i.e. abnormal vessel wall) and abnormalities in platelet and haemostatic variables (i.e. abnormal blood constituents). This review is to summarise the evidence so far for the role of coagulation and fibrinolytic components, platelets and inflammation (that is blood constituents) in the generation of the prothrombotic state in AF, with particular focus on the endothelium and AF.

The prothrombotic state in AF

Atrial fibrillation (AF) is the commonest sustained cardiac rhythm disorder which is associated with a high risk of mortality and morbidity from stroke and thromboembolism. The precise mechanisms by which AF causes thromboembolism and subsequent cerebrovascular events have yet to be fully elucidated.

Over 150 years ago, Virchow stated that for thrombus formation there should be abnormalities in flow, abnormalities in the vessel wall and abnormalities in blood constituents (30). Indeed, the mechanisms of thrombogenesis in AF can be discussed by reference to Virchow’s triad. We increasingly recognise that AF results in abnormal flow conditions with loss of atrial contractility and an irregularly irregular cardiac output, (i.e. ‘flow abnormalities’), as well as structural heart disease with endocardial damage (i.e. ‘abnormal vessel wall’) and abnormalities in platelet and haemostatic variables (i.e. ‘abnormal blood constituents’), thus fulfilling the three components of Virchow’s triad (3).

This review is to summarise the evidence so far for the role of coagulation and fibrinolytic components, platelets and inflammation (that is ‘blood constituents’) in the generation of the prothrombotic state in AF, with focus on the endothelium and AF.

Left atrial thrombus and stasis (abnormal blood flow)

Stroke in AF has been postulated by many, to be a thromboembolic event of cardio- genic origin. Certainly, the presence of thrombus in the left atrial appendage (LAA) has been demonstrated in patients with acute AF presenting with stroke, which supports this hypothesis. However, LAA thrombus can only be seen in approximately 21–23% of this population (48), and so we are left with the following question: what is the source of thrombus in the other AF patients who present with stroke, or is it simply that those in whom thrombus is not apparent have the clot that was once in their left atrium lodged in their cerebrovascular tree?

Stasis of blood in the left atrium of patients with AF is visually demonstrated by the presence of swirling spontaneous echo contrast (SEC) on transoesophageal echocardiography (seen as ‘smoke’ in the left atrium), and by demonstration of reduced LAA Doppler flow velocities (8). The presence of SEC has been correlated with outcomes in AF (28), and existence of LA thrombus, SEC and reduced LAA velocities have all been associated with clinical risk factors for thromboembolism in AF patients (23). However, although left atrial stasis and subsequent thrombus formation may be one source of thromboembolism in AF this still does not seem to provide a complete explanation.

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Abnormal blood constituents

AF has been associated with a prothrombotic state by the demonstration of circulating levels of markers indicative of increased thrombus turnover. It is well-established that AF is associated with abnormal haemostatic constituents indicative of thrombogenesis (3), the generation of which would appear to be multifactorial.

Certainly, there are reports of coagulation cascade activation and abnormal fibrinolytic activity in AF. For example, Gustaffson et al demonstrated the presence of haemostatic abnormalities in the plasma of patients with non-valvular AF when compared to sinus rhythm controls, and associated these abnormalities with stroke (18). Also, Kumagai et al. reported that haemostatic abnormalities were present in AF compared with controls who were in sinus rhythm, and that the presence of associated organic heart disease was not associated with any significant difference in fibrin D-Dimer levels (a marker of thrombogenesis), therefore suggesting the hypercoagulable state was associated with AF per se rather than its risk factors (25). Further to this, the high D-Dimer levels associated with AF seem to be reduced by both anticoagulation (29) and by cardioversion to sinus rhythm (31).

Increased thrombogenesis has been further demonstrated by increased prothrombin fragments 1 and 2 (F1+2) and thrombin-antithrombin III complex (TAT) levels as indicators of increased thrombin generation in AF patients (1), and it has been shown that F1+2 are particularly raised in AF patients with stroke, and TAT levels are particularly raised in AF patients with SEC when compared to sinus rhythm in a cross-sectional study of patients with a clinical diagnosis of heart failure (47). A study by Heppel et al also reported that patients with AF who had SEC on transoesophageal echocardiography had higher concentrations of TAT, D-Dimer and VWF associated with haemostasis, and importantly, peripheral plasma markers were also associated with the presence of LA thrombus (21).

Following this, Mondillo et al. have shown a positive linear correlation between left atrial volume and plasma fibrinogen levels alongside the endothelial markers, soluble thrombomodulin and von Willebrand factor, supporting the hypothesis that the atria may be the origin of haemostatic abnormalities observed in AF, therefore may also still be the origin of thromboembolism (40). However, haemostatic markers failed to correlate with outcome in AF in prospective studies of fibrinogen and F1+2 levels in AF patients from the Stroke Prevention in Atrial Fibrillation (SPAF) study cohort (12), and F1+2 appeared to have an association with other cardiovascular risk factors rather than future stroke in these patients. However, all participants were receiving anti-thrombotic therapy at the time, which could have interfered with this result.

The first paper showing the prognostic value of a plasma biomarker in AF was by Conway et al, who reported that raised plasma VWF levels were predictive of subsequent stroke or vascular events (7). Subsequently, high D-Dimer levels have been shown to predict thromboembolic events in patients with AF during anticoagulation (52).

However, although thrombotic indices confirm the association of AF with an intravascular environment in which there is an increased tendency to clot formation – and may have prognostic implications – the origins of this coagulation activation have had to be further explored by examination of the different components which may contribute to this prothrombotic state. Recent interest has been directed towards inflammation – as reflected by inflammatory cytokines, such as interleukins and C-reactive protein – ‘driving’ the prothrombotic state in AF, but further work is ongoing in this important area.

Fibrinolytic function

Usually when thrombus is formed, the fibrinolytic system is also activated thereby limiting thrombus propagation, as well as lysing fibrin clot. Therefore, where there is thrombus, one would expect indices of fibrinolytic system to be increased reflecting increased fibrinolytic function. However, hypofibrinolysis has been suggested in several studies in AF by increased levels of PAI (plasminogen activator inhibitor) (46), high t-PA (tissue plasminogen activator) Ag (39, 40) or tPA-PAI complex levels (46) (the majority of which is inactive), and even high lipoprotein (a) levels have been associated with thromboembolism in AF (22, 37). Data from the SPAF study showed plasminogen inhibitor-1 and t-PA complexes (PAP) levels (either due to increased plasmin formation, or as the complex is inactive, associated with decreased fibrinolysis) to be highest in those with recent onset AF, heart failure or older patients, suggesting levels may indicate higher embolic risk (11). Of course, decreased fibrinolytic function could contribute to a thrombus-forming environment but also could be a result of co-existent risk factors, endothelial dysfunction/damage or inflammation, or even a combination of all of these, rather than itself be a cause of stroke and thromboembolism in AF.

Endothelial damage

Several hypotheses have been suggested in AF, as to how the endothelium could become dysfunctional or damaged. For example, the endothelium could be rendered dysfunctional in AF, by a localised mechanism involving blood flow stasis in the left atrium or perhaps systemically due to turbulent flow and reduction in shear stresses on the vessel wall, or perhaps simply by associated cardiovascular risk factors. It may eventually be found that all of these mechanisms contribute to endothelial dysfunction in AF and through this contribute to an increased thrombotic tendency. Also, there is certainly indirect evidence of endothelial dysfunction in AF by the demonstration of changes in levels of circulating plasma markers of endothelial origin, compared to healthy/sinus rhythm controls, which needs further exploration.

VWF

Von Willebrand factor (VWF) has consistently been shown to be raised in patients with AF (13, 18, 29, 33, 34, 40). As early as 1991, Gustaffson et al. demonstrated hae-
mostatic abnormalities in association with non-valvular AF in patients with or without a previous stroke which were significantly different from sinus rhythm controls with or without a history of ischaemic stroke (18). In this study, as well as VWF, platelet factors b-thromboglobulin and platelet factor 4 were raised, as were factor VIII:C, fibrinogen and D-dimer levels. Lip et al. (29) also showed that VWF levels are increased in AF patients and that this was independent of the underlying cause of AF or the presence of structural heart disease. This increase in VWF was not altered by warfarin therapy, and this was replicated in a later study, although fibrinolytic indices were reduced by full dose warfarin therapy (35), implicating increased VWF as a cause rather than a consequence of thrombus turnover in AF.

Raised VWF is also seen in the left atrial blood samples of patients with AF associated with mitral stenosis (53) and VWF levels were increased in AF patients undergoing percutaneous balloon mitral valvuloplasty for mitral stenosis, along with markers of platelet activation (17). The increases in VWF probably occurred as a result of the frank endothelial damage (16) and increased local endothelial tissue expression of VWF (25), that have been demonstrated. Importantly though, these type of studies have also demonstrated that coagulation markers measured in peripheral samples reflect levels those in the left atrium (32), thereby enabling further studies of VWF in AF patients using peripheral sampling.

It is still uncertain as to whether increases in VWF are from the fibrillating atria or are from the vascular endothelium as a whole, but increased VWF expression has been demonstrated by immunohistochemical means in endocardial cells from the atrial appendage of AF patients (15). As there was noted to be a significant correlation between immunohistochemical grade for VWF and the degree of platelet adhesion in non-warfarinised patients, this was suggested as an important mechanism for intraatrial thrombus formation in AF.

However, although further investigators have also found raised VWF levels in AF, in the Framingham Offspring study the difference in VWF levels in chronic AF compared with healthy controls was no longer significant when the patients were stratified according to other cardiovascular disease and risk factors (13). This could be due to the relatively small number of chronic AF patients in the study, although it did lead the investigators to suggest that the changes in haemostatic variables seen in AF are a result

<table>
<thead>
<tr>
<th>authors, ref.</th>
<th>study</th>
<th>VWF levels (IU/ml) in SR (mean)</th>
<th>AF (mean)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gotfarsan et al. Stroke 1990; 21: 47–51</td>
<td>comparison of haemostatic markers in non valvular AF and sinus rhythm patients with or without prior stroke</td>
<td>20 NVAF + CVA 20 NVAF + no CVA 20 SR + CVA 40 healthy controls</td>
<td>with CVA 1.29 (1.00–1.68) healthy 1.45 (1.19–1.70)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Lip et al. Br Heart J 1995; 73: 527–533</td>
<td>comparison of fibrinogen, VWF and D-dimer levels in chronic AF vs. SR patients, plus effect of aspirin or warfarin</td>
<td>87 chronic AF 158 controls</td>
<td>105 (80–147)</td>
<td>152 (108–198)</td>
</tr>
<tr>
<td>Yoshimoto et al. JACC 1995; 25: 107–112</td>
<td>comparison of haemostatic markers between intravascular sites in patients with mitral stenosis, and comparison of peripheral VWF levels with healthy controls</td>
<td>12 mitral stenosis (11 with AF) 15 normal</td>
<td>99 ± 7**</td>
<td>168 ± 25**</td>
</tr>
<tr>
<td>Mondillo et al. Int J Cardiol 2000; 75: 227–232</td>
<td>comparison of markers of haemostatic and endothelial function in atrial fibrillation versus controls</td>
<td>45 chronic AF 35 controls</td>
<td>93.44</td>
<td>164.04</td>
</tr>
<tr>
<td>Li-Saw-Hee et al. Eur Heart J 2001; 22: 1741–1747</td>
<td>comparison of haemostatic markers in chronic (paroxysmal, persistent and permanent) atrial fibrillation compared with healthy controls</td>
<td>23 paroxysmal 23 persistent 23 permanent 20 controls</td>
<td>101 ± 30</td>
<td>130 ± 34 106 ± 26 143 ± 47</td>
</tr>
<tr>
<td>Feng et al. Am J Cardiol 2001; 87: 168–171</td>
<td>haemostatic state and atrial fibrillation (Framingham Offspring study)</td>
<td>47 AF 167 controls</td>
<td>127.6 ± 45.9**</td>
<td>142 ± 46.2**</td>
</tr>
</tbody>
</table>

*versus healthy controls, Figures in brackets denote inter-quartile range, **VWF Ag expressed as % of that found in pooled normal plasma, expressed as mean ± standard deviation; ***note this difference was no longer significant after central subjects matched for other cardiovascular risk factors.

Tab. 1 Examples of VWF levels found in early AF studies
of the frequently associated cardiovascular co-morbidities rather than AF per se.

Whether raised VWF levels are due to the AF itself, or coexistent cardiovascular risk factors remains a matter of debate, but the association of VWF with both outcome in AF and with other stroke risk factors in AF patients (6, 7), supports the importance of endothelial dysfunction as a fundamental part of thrombogenesis and stroke risk in this condition.

Other endothelial markers have also been tested in AF but with somewhat variable results.

**Soluble thrombomodulin**

Soluble thrombomodulin (sTM) has been measured as a marker of endothelial dysfunction in AF by several groups, but so far with conflicting results. As eluded to earlier this could perhaps be explained by low levels of sTM being seen in endothelial dysfunction due to down-regulation of expression, as compared with the high levels seen in endothelial damage.

Li-Saw-Hee et al. (32) demonstrated lower levels of sTM in cardiac and peripheral plasma samples of patients with AF and mitral stenosis prior to valvuloplasty in comparison with sinus rhythm controls. The lower levels of sTM seen in this group have been postulated to be associated with recent discontinuation of warfarin therapy, although it is possible that this change could be a true reflection of dysfunctional endothelial cell presentation of sTM in the absence of any endothelial damage. This is in contrast with the findings of Mondillo et al. (40) who found that levels of sTM were increased in the peripheral samples of patients with lone chronic non-rheumatic AF when compared with controls. These patients were not on warfarin and could possibly explain the different findings. Another possibility is the presence of endothelial damage in these lone AF patients who may have undiagnosed atherosclerotic disease at their mean age of 67 years, which could be an aetiological factor in this group when compared with the valvular AF group previously studied. Thus, the role of sTM as an endothelial marker in AF is unclear.

**E-selectin**

E-selectin (sE-sel) is a specific endothelial cell surface leucocyte adhesion molecule thought to be indicative of endothelial cell activation (27). A related group have reported sE-sel levels to be similar in AF to controls with similar cardiovascular co-morbidity (45), but assessment of endothelial activation by measurement of E-sel in lone AF or subsets of AF patients has not yet been undertaken.

**Nitric oxide**

NO concentrations (as measured by nitrate/nitrite product, NOx), and cyclic GMP levels in platelets (as an indicator of NO activity) have been shown to be reduced in patients with AF (38). Along side their demonstration of haemostatic abnormalities seen in AF, the reduced NOx concentration caused by endothelial dysfunction, was suggested to be the mechanism by which AF could lead to haemostatic abnormalities and subsequent thromboembolic risk.

The same investigators showed that the plasma NOx concentration decreases, whilst expression of P-selectin of platelets increase, shortly after the onset of AF in a canine model (38). These same changes were subsequently demonstrated to be significantly different between patients in AF compared with sinus rhythm controls. Correlation of these findings with the high incidence of silent cerebral infarction (92%) in the AF study group supports the relevance of these findings with regards to high thromboembolic risk. In a study of left atrial appendage tissue, Fukuchi et al. (15) demonstrated the importance of VWF in the endocardium for the degree of platelet adhesion/thrombosis, and also showed that endothelial nitric oxide synthase (eNOS) was deficient in these areas. eNOS down-regulation and a subsequent decrease in NO production is therefore also likely to be an important step in thrombogenesis in AF.

Flow-mediated dilatation measured by brachial ultrasound (9), as a surrogate measure of nitric oxide release by the endothelium, has been measured in AF (14). In our study of 40 patients with chronic stable AF, baseline brachial artery diameter did not differ significantly between AF and healthy control subjects, but FMD was significantly impaired in AF patients. Of note, there was no significant difference in endothelium-independent (GTN-induced) dilatation between AF and controls, and only AF and male sex were independent predictors of impaired FMD on stepwise multiple regression analysis (p < 0.0001). Thus, such endothelial dysfunction may contribute to pathophysiology of thromboembolism in AF.

Also, changes in forearm blood induced by acetylsalicylic acid have been shown to be reduced when compared with nitroglycerin response in subjects with AF using plethysmography, again indicating impaired endothelial function in AF (50, 51). Although NO has now been measured in acute AF and the effects of DC cardioversion on NO production reported (42), the role of total body nitric oxide production measured by NOx in chronic AF has not been examined. The recent role of inflammation, as indicated by raised CRP and Interleukin-6, in association with AF (5) may make interpretation of NOx levels somewhat complex due to the theoretical contribution of inducible nitric oxide synthase, although this does need to be explored.

**Tissue factor**

Although strictly speaking not an endothelium-specific marker, tissue factor (TF) is predominantly found in the sub-endothelium therefore it may be exposed during endothelial damage. Circulating TF levels have been shown to be increased in association with VEGF in AF patients by Chung et al. (4), perhaps suggesting an endothelial origin for both of these molecules. As TF has recently been localised to the atria of patients with AF (41), its association with left atrial thrombus could indicate a key role of TF in the generation of a hypercoagulable state in AF as well as confirming localised abnormal endothelial expression of TF in these patients.
Interventions on the endothelium in AF

Cardioversion by electrical or pharmacological means has been used to restore sinus rhythm in AF patients. AF can be associated with significant symptoms, haemodynamic and thromboembolic consequences, therefore a return to sinus rhythm has long been the goal in AF management on the assumption that restoration of sinus rhythm is physiological and therefore preferable to continuing arrhythmia. Certainly, conversion of AF to sinus rhythm provides a return of atrial transport (43), which may have an important contribution to stroke volume, and short term studies show an improvement in exercise capacity and haemodynamic improvements post-cardioversion (19, 44). Further to this, there was the hope that the association of AF with a prothrombotic or hypercoagulable state, (with abnormalities in haemostasis, platelets and endothelial function that are unrelated to underlying structural heart disease or aetiology of AF) may be improved by a return to sinus rhythm.

However, large studies examining the strategy of rhythm control versus rate control in the management of AF have not yet proven the benefit of a return to sinus rhythm (49). If anything, rhythm control appeared to be associated with a greater risk of thromboembolism and drug adverse effects. Although these trials have been widely criticised, it is of note that strokes tended to occur in the rhythm control group when AF was recurrent or when patients were under-anticoagulated (10).

Conclusion

As summarised, of the many indices of a prothrombotic state in AF, plasma VWF – an index of endothelial damage/dysfunction – is consistently raised in AF and high levels are prognostically linked to stroke and vascular events (7). Whether VWF is released from the damaged atrial endocardia (16) – which has increased endothelial tissue expression of VWF (26) and tissue factor in non-valvular AF (41) – acting as a cardio-

genic thromboembolic source, or whether the whole of the vascular endothelium is dysfunctional is unclear, but endothelial damage/dysfunction has been purported to have an important role in the pathophysiology of thromboembolism in AF.

Some endothelial abnormalities observed with AF appear to normalise with a return to sinus rhythm. For example, Nikitovic et al. (24) found raised VWF and depressed nitric oxide levels in AF patients, which normalised at 30 days following DC cardioversion (42). Similarly, Takahashi et al. found a reduced forearm-blood flow response to acetylcholine (as an indicator of endothelial nitric oxide bioavailability) in AF, but vascular endothelial function was significantly improved by a return to sinus rhythm after just 1 day (51). More recently, Kato et al. studied hepatocyte growth factor – as another marker of vascular endothelial injury – and showed this to be raised in AF compared with controls with, following cardioversion, decreased levels seen at seven days and a return to control values at one month in those maintaining sinus rhythm, alongside normalisation of endothelial-dependent forearm blood flow (24). Thus, these studies show that endothelial function seems to improve by conversion from AF to sinus rhythm.

So why do these potential benefits of cardioversion – symptoms, haemodynamic, endothelial function, etc – then not translate into long term benefit in the large randomised trials? One explanation could come from studies which have shown little or no change in endothelial markers when AF is cardioverted, either electrically or pharmacologically, suggesting that sinus rhythm does not necessarily completely restore endothelial homeostasis (20, 36). For example, Li-Saw Hee et al. (33) did not find any significant changes in plasma VWF following cardioversion, with no relationship to the return of atrial systolic function. Also, even when sinus rhythm appears to be maintained, thromboembolic events seem to occur predominantly in patients previously known to have AF in association with other stroke risk factors, as well as when there is subtherapeutic (or no) anticoagulation (10). Furthermore, even when there is no identifiable pre-existing left atrial thrombus, thromboembolic events have still been reported to occur in AF (48). Evidence of direct endothelial damage can certainly be demonstrated (perhaps not surprisingly) in the setting of pulmonary vein isolation for AF, which could act as a thromboembolic stimulus in a catheter ablation setting (2), but this does not necessarily translate to stroke risk in the every-day clinical management strategy of most AF patients. Indeed, pre-existing endothelial disruption/per-turbation – often in association with co-morbidities that are not going to be corrected completely simply by a return to sinus rhythm – are likely to contribute to the thromboembolism risk burden in AF.

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