Mechanisms of thrombogenesis in atrial fibrillation: Virchow's triad revisited

Timothy Watson, Eduard Shantsila, Gregory Y H Lip

Atrial fibrillation is the most common sustained cardiac arrhythmia, which is associated with a high risk of stroke and thromboembolism. Increasing evidence suggests that the thrombogenic tendency in atrial fibrillation is related to several underlying pathophysiological mechanisms. Abnormal changes in flow are evident by stasis in the left atrium, and seen as spontaneous echocontrast. Abnormal changes in vessel walls—essentially, anatomical and structural defects—include progressive atrial dilatation, endocardial denudation, and oedematous or fibroelastic infiltration of the extracellular matrix. Additionally, abnormal changes in blood constituents are well described, and include haemostatic and platelet activation, as well as inflammation and growth factor changes. These changes result in the fulfilment of Virchow's triad for thrombogenesis, and accord with a prothrombotic or hypercoagulable state in this arrhythmia. In this Review, we present an overview of the established and purported mechanisms for thrombogenesis in atrial fibrillation.

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University Department of Medicine, City Hospital, Birmingham, UK (T Watson MRCP, Prof G Y H Lip FRCP)**; and Republican Research and Practical Centre Cardiology, Minsk, Belarus** (E Shantsila MD)

Correspondence to: Prof Gregory Y H Lip, University Department of Medicine, City Hospital, Birmingham B18 7QH, UK
g.y.h.lip@bham.ac.uk

Introduction

The association between atrial fibrillation and the risk of stroke and thromboembolism has long been recognised. The pathogenesis of thrombus formation (thrombogenesis) in atrial fibrillation is multifactorial and is not only related to stasis in a poorly contractile left atrium. Indeed, there is an increasing body of evidence to support the presence of a prothrombotic or hypercoagulable state.[1]

More than 150 years ago, Rudolf Virchow proposed a triad of events needed for thrombus formation—ie, abnormal changes of the vessel wall, blood flow, and blood constituents.[2] In the 21st century, we now recognise Virchow's triad as: endothelial or endocardial damage or dysfunction (and related structural abnormal changes); abnormal blood stasis; and abnormal haemostasis, platelets, and fibrinolysis. Extensive abnormal changes of these variables are clearly evident in atrial fibrillation. Thus, atrial fibrillation could in fact drive a prothrombotic or hypercoagulable state, by virtue of its fulfilment of Virchow's triad for thrombogenesis.[3]

Anatomical and structural considerations

Attached to each atria is a blind-ended passage known as an appendage. The left atrial appendage (LAA) is long with a narrow inlet, thereby predisposing to blood stasis. Thus, the LAA is the most common site of intra-atrial thrombus formation, not only in atrial fibrillation, but also in patients with sinus rhythm.[4,5]

Changes in the dimensions of the left atrium and LAA occur as a consequence of atrial fibrillation, with some correlation to subsequent thromboembolism. Detailed descriptions of endothelial damage in the context of atrial fibrillation are well described and can be visualised by scanning electron microscopy, especially within the appendages. Goldsmith and colleagues have reported more severe endocardial changes in the LAA than in the right-atrial appendages,[6] especially in atrial fibrillation (compared with sinus rhythm) and in mitral stenosis (compared with mitral regurgitation). Similarly, Masawa and co-workers[7] have described a "rough endocardium" with a wrinkled appearance attributable to oedema and fibrinous transformation; small areas of endothelial denudation and thrombotic aggregation have also been noted in patients with atrial fibrillation and cerebral embolism.

Subsequent work has confirmed that these changes are present, even in those without valvular heart disease.[8] Other changes, including myocytic hypertrophy or necrosis and a mononuclear cell infiltrate, are also evident.[9] These structural changes (with or without electrical changes) could explain the delay in return of atrial systole after successful cardioversion.[10,11] The occurrence of such cardiac stunning highlights the importance of adequate anticoagulation after restoration of sinus rhythm.[12]

However, not all structural changes in atrial fibrillation are cardiac. Complex aortic plaque identified by transoesophageal echocardiography (TEE) is common and occurs in up to 57% of patients with atrial fibrillation, of whom about 25% have complex plaque (ie, thicker than 4 mm and with ulceration, pedunculation, or mobile elements).[13] Presence of complex plaque on the descending aorta is a risk factor for stroke.[14] Aortic plaque

Search strategy and selection criteria

We did a comprehensive literature search by using electronic bibliographic databases (ie, Medline, Embase, DARE, Cochrane database), scanning reference lists from included articles, and hand searching abstracts from national and international cardiovascular meetings. We used search terms including "atrial fibrillation", "hypercoagulability", "inflammation", "anti-thrombotic treatment". Bibliographies of all selected articles and review articles were reviewed for other relevant articles. Finally, the supplements of major journals were hand searched to identify relevant abstracts that had not been published as peer-reviewed articles. If necessary, study authors were contacted to obtain further data.

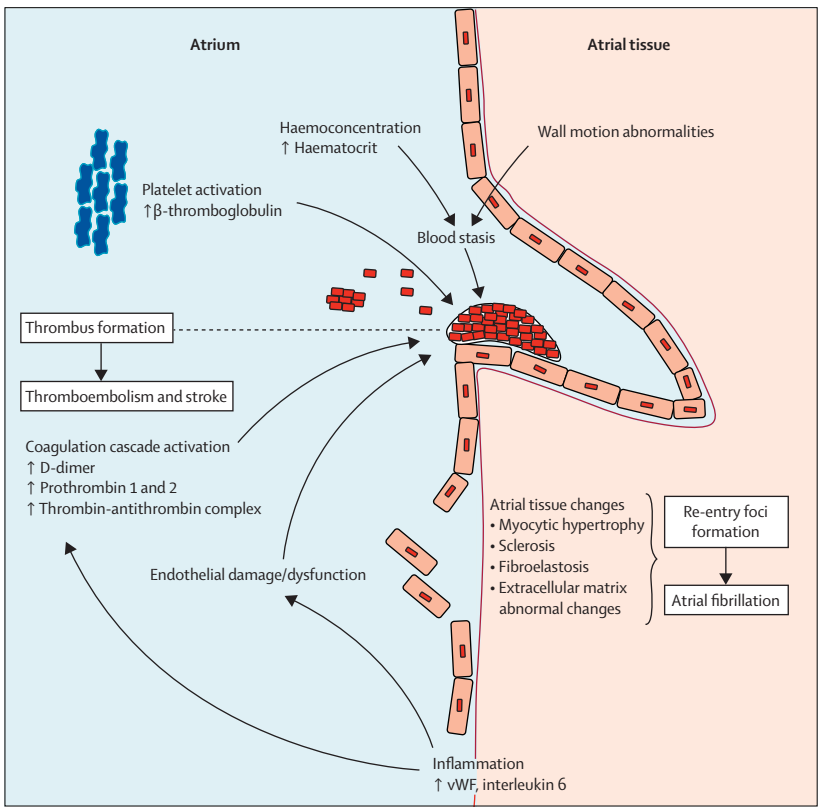

Figure 1: **Components of Virchow's triad for thrombogenesis in atrial fibrillation**
Abnormal changes shown in the vessel wall (eg, atrial tissue changes, endothelial damage and dysfunction), in flow (stasis—eg, in the left atrial appendage), and in blood constituents (eg, haemoconcentration, platelets, coagulation cascade activation, inflammation); all factors contribute to propensity for thrombus formation (thrombogenesis) in atrial fibrillation. vWf=von Willebrand factor.

simply aids identification of patients who are at high stroke risk by virtue of the presence of associated vascular risk factors or atherothrombotic disease, in addition to atrial fibrillation.

Extracellular matrix turnover could be implicated in the structural changes associated with atrial fibrillation. The extracellular matrix provides a support scaffolding for myocytes, maintaining the structural and geometrical integrity of the heart.[15] Disruption of the extracellular matrix has the potential not only to result in conduction defects (perpetuating atrial fibrillation), but also to induce fibrosis and infiltration of the endocardium, and thereby promote thrombogenesis. Several studies have shown that patients with atrial fibrillation have altered amounts of collagen degradation products and impaired matrix degradation, with abnormal plasma concentrations of various matrix metalloproteinases (MMPs), their inhibitors (tissue inhibitor of MMPs [TIMPs]), and various growth factors (eg, transforming growth factor β1) reported.[16–18] These proteins are important in the breakdown of various collagens and hence their regulation is key to ensuring healthy matrix turnover.

Evidence suggests that abnormal changes in the extracellular matrix are not related to the presence of atrial fibrillation in itself, but are probably a consequence of various coexisting comorbidities (eg, hypertension). Nevertheless, MMPs and TIMPs could have a link with the prothrombotic state, as exemplified by a correlation with prothrombin fragments 1 and 2, markers of thrombogenesis.[17] Further studies have identified disruption of other extracellular matrix components, although most have focused on these factors as a cause for the arrhythmia or explanation for remodelling and chamber dilatation.[19–22] One study suggested that some of the changes in MMPs were due to concomitant mitral valve disease,[21] whereas another reported changes in the ventricular myocardium, albeit to a lesser extent.[22] Similarly, in patients with ventricular dysfunction (a potent risk factor for atrial fibrillation), various studies have also shown striking atrial structural changes.[23,24]

Abnormal blood stasis

In addition to stasis consequent on the failure of atrial systole, the presence of non-valvular atrial fibrillation seems to promote progressive left atrial (LA) dilatation,[25] thus amplifying the potential for stasis. In the presence of mitral stenosis, LA dilatation is increased and leads to further stasis and propensity to thrombosis.[26] The contribution of LA dilatation to thrombogenesis (at least, in non-valvular atrial fibrillation) is indicated by the finding that atrial size corrected for body surface area is an independent risk factor for stroke.[27,28]

The contribution of valvular heart disease to thrombogenesis in atrial fibrillation cannot be ignored. In mitral stenosis, up to 75% of patients with cerebral emboli on computed tomography or autopsy are identified to have atrial fibrillation, presumably due to alterations in LA emptying and transmitral flow.[29] By contrast, moderate-to-severe (non-rheumatic) mitral regurgitation seems to reduce the risk of stroke with atrial fibrillation.[30] Defining patients with atrial fibrillation and mitral valve disease who are at the greatest risk of stroke has proved complex. The risk of emboli increases with age and in individuals with a lower cardiac index, but seems to correlate poorly with clinical classification or mitral valve area. Studies assessing the degree of LA dilatation have also proven inconsistent. However, an initial embolic event is highly predictive for subsequent or recurrent thromboemboli.[31]

Abnormal stasis in the LA and LAA can be visualised on TEE with spontaneous echo contrast (SEC) or pulsed-wave doppler during paroxysms of atrial fibrillation.[32–35] In sinus rhythm, a quadriphasic pattern of blood flow can be seen in the LAA, affording minimum blood stasis.[36] This pattern in blood flow is thought to be related to the intimate yet slightly delayed relations between atrial and ventricular passive and active filling. In atrial fibrillation, SEC has been shown to independently predict increased risk of thromboembolism.[37]

SEC is thought to be related to increased interaction between fibrinogen and erythrocytes and seems to relate

to the relative concentrations of each, with more fibrinogen needed to induce the same effect at lower haematocrits.[38–40] Since some patients with atrial fibrillation can be intravascularly deplete (for example, as a result of co-prescription of diuretics), this finding could contribute to the increased stroke rate seen in this patient population.[41] Crucially, SEC is also highly dependent on flow rate and thus more likely to occur in patients predisposed to stasis, as in atrial fibrillation.

SEC can also be seen after restoration of sinus rhythm and could occur in up to 37% of this cohort at 3 months.[42] With additional stroke risk factors, this proportion is higher still, illustrating the need for continuing anticoagulation despite apparent maintenance of sinus rhythm.

Abnormal blood constituents

The main intravascular promoters of thrombogenesis are platelets and the various proteins of the coagulation cascade. In atrial fibrillation, abnormal changes in both these promoters and other blood constituents (eg, inflammatory cytokines, growth factors) are evident, thereby completing Virchow's triad.

Abnormal changes in coagulation

Abnormal haemostasis and coagulation are well described in atrial fibrillation (figure 1, webtable 1). In particular, increased fibrin turnover has been reported in patients with acute onset or chronic atrial fibrillation.[43–49] These changes initially seemed to be unrelated to the cause of atrial fibrillation or structural heart disease.[48,49] However, abnormal concentrations of prothrombotic indices (eg, prothrombin fragments 1 and 2 and thrombin-antithrombin complexes) are more prominent in patients with stroke who have atrial fibrillation than in those who have sinus rhythm,[50] as well as in patients with atrial fibrillation and many stroke risk factors (eg, diabetes plus heart failure) compared with either risk factor alone.[51–53] Furthermore, some prothrombotic indices are abnormal in the patients with atrial fibrillation only[54,55] and in those with paroxysmal atrial fibrillation.[56] Notably, some markers have been proposed as suitable candidates to refine various stroke risk stratification schema, many of which are reasonably able to identify patients at low risk or high risk of stroke, but poor at identifying patients at moderate risk.[57]

An association between various prothrombotic indices, stasis, and intracardiac thrombus has been described.[58,59] In one study, congestive cardiac failure, a history of recent embolus, and fibrin D-dimer were shown to independently predict the presence of LAA thrombi on TEE, leading the researchers to conclude that D-dimer could be useful in predicting the absence of LAA thrombi.[59]

The prothrombotic state also correlates with the degree of LAA dysfunction.[60,61] Furthermore, a relation to TEE indices of stroke risk has been described. For example, SEC that is visible during TEE shows a significant correlation to prothrombin fragments 1 and 2, fibrinopeptide A, and thrombin-antithrombin III complex in non-valvular atrial fibrilllation.[62,63] Patients with atrial flutter and impaired LAA function (shown by pulsed-wave doppler) have increased amounts of of D-dimer and β-thromboglobulin.[63] In accordance with clinical data suggesting that mitral regurgitation protects against stroke in atrial fibrillation, a greater degree of mitral regurgitation is associated with reduced coagulation activity as estimated by fibrin D-dimer amounts,[64] highlighting the important contribution of stasis.

Anticoagulant treatment has been shown to reduce concentrations of some prothrombotic markers.[48,65,66] This finding is true even with low-intensity anticoagulation (international normalised ratio [INR] 1·5–1·9), which has been shown to suppress prothombin fragments 1 and 2 and D-dimer.[67] Notably, some indices of hypercoagulability could be useful in investigating the efficacy of antithrombotic treatment for atrial fibrillation. For example, the Atrial Fibrillation, Aspirin, AntiCoagulation (AFASAK-2) substudy[68] reported that only dose-adjusted warfarin (INR 2–3) had an effect on the amounts of prothrombin fragments 1 and 2 after 3 months' treatment. Fixed low-dose warfarin, combined low-dose warfarin and aspirin, or aspirin alone had little effect on prothrombin fragments. Similarly, fixed low-dose warfarin or aspirin-warfarin combination treatment did not substantially reduce other markers of thrombogenesis in atrial fibrillation, whereas dose-adjusted warfarin did.[69] Additionally, warfarin greatly decreases plasma concentrations of coagulation factor-related prothrombotic indices (more so than those of platelet-related indices), which implies that activation of the coagulation cascade, rather than platelets, is key to the excess thromboembolic risk in atrial fibrillation.[70,71] This finding is consistent with reports that warfarin (as a modulator of the coagulation cascade) is more efficacious than aspirin (a platelet inhibitor) in thromboembolic prophylaxis in atrial fibrillation.

See Online for webtable 1

In chronic atrial fibrillation, D-dimer amounts remain in the same range over time and seem to be a useful parameter for assessing the degree of hypercoagulability irrespective of patient age.[44] Combined with clinical risk factors, D-dimer has also been shown to predict subsequent thromboembolic events in patients with non-valvular atrial fibrillation, including those already receiving treatment with warfarin.[72–74] The application of D-dimer seemed to be especially important in patients without conventional risk factors for stroke (eg, age, cardiomyopathy, previous stroke), whereby a low amount of D-dimer implied a low risk of stroke (0·7% per year). Conversely, in the same patient group, the stroke rate rose to 3·8% per year when D-dimer was measured. In patients with clinical risk factors for stroke, the event rate was less than 5%, irrespective of D-dimer concentration. In another study of patients with chronic atrial fibrillation,

both D-dimer and age were important predictors of mortality. High amounts of D-dimer during treatment with oral anticoagulants was also a predictor of combined cardiovascular events.[74]

Thus, D-dimer could be useful as a screening method to identify those patients with atrial fibrillation at low risk of intracardiac thrombus who can then be safely cardioverted without anticoagulation. Notably, Somlói and colleagues[75] have suggested that D-dimer measurements compare favourably with the use of a TEE-guided strategy with a negative predictive value of 98%.

von Willebrand factor (vWf)

Further insight into the hypercoagulable state in atrial fibrillation is provided by studies of vWf, which is a well-established index of endothelial damage and dysfunction. Raised vWf concentrations independently predict presence of LAA thrombus in atrial fibrillation.[58] Furthermore, increased LAA endocardial expression of vWf has been described,[76] especially in those with an overloaded appendage, which seems to correlate with the presence of adherent platelet thrombus. Furthermore, increased expression of vWf in the endocardium has been shown to associate with enlarged LA dimensions in mitral valve disease and increased myocyte diameter.[77]

Both vWf and tissue factor are overexpressed in the atrial endothelium in patients with atrial fibrillation who have a history of cardiogenic thromboembolism—specifically in the endothelial sites containing inflammatory cells and denuded endocardium, which indicate features of persistent myocarditis.[78] Plasma vWf and D-dimer are also positively correlated in patients receiving either aspirin or no antithrombotic treatment, but not in those receiving warfarin,[48] further indicating the ability of warfarin to modulate the thrombogenic process.

See Online for webtable 2

Furthermore, a positive association between atrial fibrillation and plasma vWF was seen in the Rotterdam study.[79] This relation was most apparent in female patients, which could explain the excess risk of stroke due to atrial fibrillation in women compared with men. Furthermore, plasma vWf amounts were associated with the presence of four independent risk factors for stroke (heart failure, previous stroke, age, and diabetes) and stroke risk stratification schema.[80,81] Follow-up data from this study suggests that vWf concentrations might independently predict subsequent stroke and vascular events.[81,82] However, such applications will probably be hampered by the non-specificity of vWf, concentrations of which are also increased in various other disorders.[83,84]

Abnormal changes in fibrinolysis

Few studies have focused on fibrinolytic function in atrial fibrillation. Enhanced fibrinolysis, shown by increased concentrations of tissue-plaminogen activator (t-PA) antigen and t-PA inhibitor (PAI)-1 and reduced amounts of plasmin-antiplasmin complex can be attributable to a pathophysiological response to the prothrombotic state.[85,86] However, the available data are not consistent and conflicting results have also been reported.[45]

In the Stroke Prevention in Atrial Fibrillation (SPAF) III study,[87] increased concentrations of plasmin-antiplasmin complexes were independently associated with thromboembolic risk factors such as older age (>75 years), recent congestive heart failure, decreased fractional shortening, and recent onset of atrial fibrillation. A significant correlation can be also shown between t-PA amounts and left-atrial diameter in atrial fibrillation.[45] Predictably, anticoagulation leads to some improvement in fibrinolytic markers in rheumatic atrial fibrillation.[88]

Increased amounts of t-PA and PAI-1 can indicate the coexistence of confounders, such as hypertension, heart failure, or ischaemic heart disease, all of which can cause endothelial dysfunction, damage, and inflammation. However, studies in patients with atrial fibrillation only confirm that presence of the disorder does modulate these markers.[45,85,88] Thus, the high amounts of t-PA and PAI-1 in atrial fibrillation could be a consequence of endothelial damage and dysfunction or represent systemic inflammation.[89,90] PAI-1 concentrations are also predictive of successful cardioversion,[91] and are independent predictors of the development of atrial fibrillation after cardiopulmonary bypass.[92]

It is unclear whether increased amounts of t-PA or PAI-1 in atrial fibrillation are due to endothelial dysfunction, inflammation, fibrinolysis, or vascular disease, or a combination. Nevertheless, abnormal changes in the fibrinolytic system might relate not only to thrombogenesis but also to structural remodelling of the atria, in view of the strong links to extracellular matrix turnover.

Platelets

Many studies indicate a potential role for platelets in the hypercoagulable state (webtable 2). However, the results of many of these studies have been conflicting, representing the diverse aspects of platelet physiology that have been measured and possibly confounding from interlaboratory assay variability. The available data support the notion that abnormal changes of platelets in atrial fibrillation do exist, but the relation between these measures and increased thrombotic risk remains uncertain, and many of such abnormal changes could simply indicate underlying vascular comorbidities.

For example, Choudhury and colleagues[93] recently showed that patients with atrial fibrillation had far higher amounts of platelet microparticles and soluble P-selectin than healthy controls in sinus rhythm, but no difference was seen between patients with atrial fibrillation and disease-matched controls, implying that the abnormal changes detected were a consequence of the underlying comorbidities rather than atrial fibrillation itself. Increased amounts of β-thromboglobulin, a platelet-specific protein that indicates platelet activation and is released from

α-granules during platelet aggregation and subsequent thrombus formation, have been shown in patients with both valvular and non-valvular atrial fibrillation compared with controls in sinus rhythm.[61,71,94–98] Substantially higher β-thromboglobulin amounts have been measured in patients with the lowest LAA flow velocities, who had greater left-atrial dimensions,[61] suggesting that platelet activation could be enhanced in patients with a greater degree of intra-atrial stasis.

Notably, antithrombotic treatment modulates only some of these abnormal changes, and Kamath and co-workers[70] did not show a beneficial effect of warfarin on plasma β-thromboglobulin concentrations. In the same study, in-vitro measures of platelet aggregation were not significantly increased in atrial fibrillation, once again questioning the importance of platelets in enhancing the thrombogenic tendency in this setting.[70] In other studies, oral anticoagulation also did not reduce platelet activation in atrial fibrillation, despite pronounced inhibition of other coagulation variables.[99,100] By contrast, aspirin has reduced concentrations of soluble P-selectin compared with warfarin in atrial fibrillation.[101] Notably, some patients given aspirin 325 mg per day still do not show complete inhibition of platelet aggregation, whereas others have hyper-aggregable platelets.[102] In view of recent interest in aspirin resistance, these findings raise the possibility of platelet-dependent mechanisms for aspirin and warfarin failure to prevent stroke in patients with atrial fibrillation.

Although combined antiplatelet treatment with aspirin and clopidogrel is more effective than aspirin monotherapy in inhibiting platelet function,[103] this strategy does not substantially modulate the markers of the coagulation cascade (eg, platelet-dependent thrombin generation, antithrombin III, thrombin-antithrombin III complex, prothrombin fragments 1 and 2) in patients with atrial fibrillation. Such data accord with clinical trials, in which combined antiplatelet treatment with aspirin plus clopidogrel was shown to be less effective than warfarin for stroke prevention in atrial fibrillation.[104] These findings are also supported by data showing that patients show changes in plasma markers of platelet function but not platelet aggregation, which are unaffected by anticoagulation with warfarin.[105]

The use of digoxin in patients with atrial fibrillation seems to be associated with platelet activation, with increased amounts of CD62P (P-selectin) expression on platelets and platelet-leucocyte conjugates, which could predispose to thrombosis and vascular events.[100] Enhancement of platelet activity and coagulability occur within 12 h of onset of atrial fibrillation,[106] whereas after restoration of sinus rhythm, substantial reduction in platelet activity is seen compared with controls at 24 h after cardioversion.[107] During radiofrequency ablation of atrial fibrillation, persistent platelet activation is reported, but is not apparent during cryoablation.[108]

Despite the presence of enhanced platelet activation in atrial fibrillation, any firm clinical evidence indicating that it directly enhances thrombotic risk is lacking. A substudy from the Stroke Prevention in Atrial Fibrillation III (SPAF-III) trial[109] recorded no association between plasma β-thromboglobulin amounts and subsequent thrombo-embolic events. By contrast, the population-based Rotterdam study[110] showed that plasma concentrations of soluble P-selectin were predictive of adverse clinical outcomes in elderly patients with atrial fibrillation.

In view of the close links between platelet activation and the atherothrombotic vascular comorbidities related to atrial fibrillation, the platelet activation seen in this arrhythmia could contribute to thrombogenesis indirectly. For example, increased expression of P-selectin on platelets associated with reduced concentrations of nitric oxide has also been shown to be a risk factor for silent cerebral infarction in patients with atrial fibrillation.[111] Moreover, raised amounts of P-selectin and CD63 have both been associated with the embolic and pre-embolic status of patients with non-rheumatic atrial fibrillation.[112]

Restoration of sinus rhythm

Some evidence suggests that activation of the coagulation system could be adversely affected by cardioversion of atrial fibrillation.[113] Electrical cardioversion has been associated with more prominent activation of the coagulation system than a pharmacological strategy.[114] One study found a positive correlation between the energy delivered for cardioversion to sinus rhythm and plasma D-dimer values on day 7.[114] Additionally, an extended duration of atrial fibrillation could lead to a more prominent hypercoagulable state (estimated by D-dimer value) after cardioversion.[115] The hypercoagulable state after cardioversion has been seen despite optimum anticoagulation with warfarin.[116] Nevertheless, patients receiving therapeutic low-molecular-weight heparin (LMWH) before cardioversion seem to have reduced hypercoagulability.[117] In atrial flutter, limited data suggest that plasma amounts of D-dimer, platelet factor 4, β-thromboglobulin, thrombin-antithrombin III complex, and prothrombin fragments 1 and 2 remain raised, but do not seem to increase further with cardioversion of this arrhythmia.[118]

What drives the prothrombotic state in atrial fibrillation?

Several mechanisms have been purported to drive the prothrombotic state in atrial fibrillation (figure 2), but recent evidence has focused on the potential role of inflammation and the release of various growth factors.

Inflammation

In atrial fibrillation, inflammation might not only result in endothelial damage, dysfunction, or activation, but also be linked directly to thrombogenesis. Increasing

evidence has supported a link between inflammation and the initiation and perpetuation of atrial fibrillation.[119–123] Furthermore, abnormal changes in systemic inflammation have been related to prothrombotic indices in atrial fibrillation, suggesting that inflammation could drive the prothrombotic state in atrial fibrillation.[119]

Although most cases of atrial fibrillation are associated with various comorbidities, many of which could also enhance the baseline inflammatory state, there may be an underlying direct link between atrial fibrillation and inflammation. Interleukin-6 concentrations are abnormal in atrial fibrillation, with some prognostic implications shown in one study.[124] Many studies have also shown that amounts of high-sensitivity C-reactive protein (hs-CRP) are greater in patients with atrial fibrillation than in controls in sinus rhythm, with a stepwise increase in hs-CRP with the transition from patient groups with an increasing burden (sinus rhythm to paroxysmal then persistent) in atrial fibrillation.[125] Raised hs-CRP amounts consistently correlate with cardiovascular risk, although not with future atrial fibrillation.[119] Also, reduced concentrations of both hs-CRP and E-selectin at baseline are associated with an increased probability of maintenance of sinus rhythm at 6 months after electrical cardioversion for atrial fibrillation, although the maintenance of sinus rhythm seems to have no effect on hs-CRP.[126] More recently, high hc-CRP amounts were shown to be predictive of mortality and vascular death in atrial fibrillation, but not stroke itself.[127]

How is inflammation linked to thrombogenesis in atrial fibrillation? Both CRP and interleukin 6 stimulate tissue factor production from monocytes in vitro.[128,129] Furthermore, interleukin 6 increases platelet production and sensitivity to thrombin,[130] stimulates transcription of fibrinogen,[131] and is linked to both endothelial activation and damage.[132,133] However, no link seems to exist between hs-CRP and thrombin-antithrombin complexes.[134] Tissue factor and high stroke risk are also independent associates of interleukin 6, whereas fibrinogen and plasma viscosity are independent associates of hs-CRP amounts.[135]

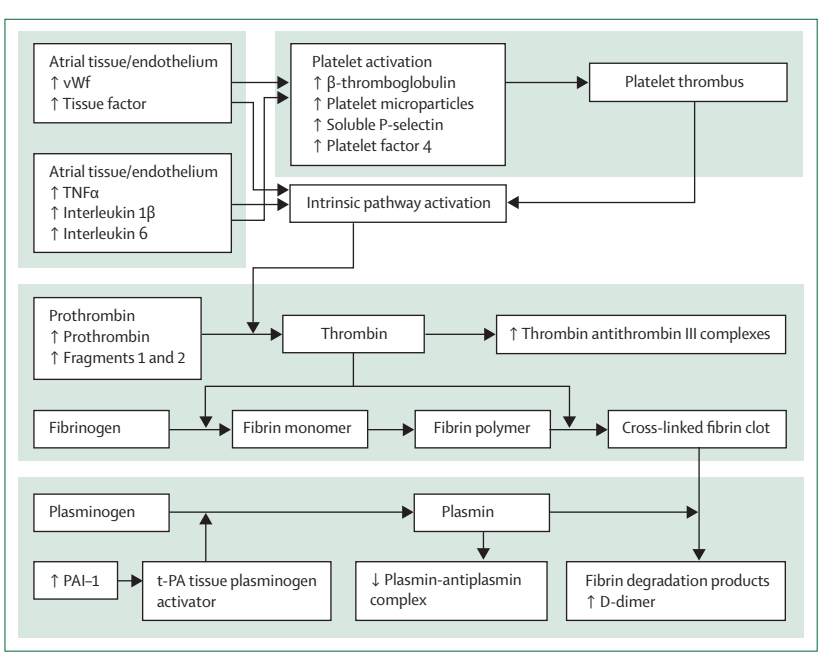

Figure 2: **Abnormal changes in coagulation during atrial fibrillation**

Growth factors

Another potential driver for thrombogenesis could be growth factors. In cancer biology, various pro-angiogenic factors, in particular vascular endothelial growth factor (VEGF), act as potent stimulants for tissue factor expression.[136] This activity could partly explain the enhanced thrombogenic risk often associated with cancer.

Various pro-angiogenic factors have been identified; concentrations of some of these factors have been shown to alter in atrial fibrillation.[137–139] VEGF is largely produced by activated platelets,[140] and results in upregulation of tissue factor mRNA production and subsequent expression of this compound on the endothelial membrane.[141] VEGF amounts are substantially increased in both persistent and permanent atrial fibrillation, with a corresponding increase in tissue factor.[137] Additionally, raised serum concentrations of transforming growth factor-β1[138] and angiopoetin 2 (but not angiopoetin 1)[139] are also recorded in atrial fibrillation, showing the depth and complexity of modulation of growth factor amounts. Although the requirements for enhanced angiogenesis in atrial fibrillation are unknown, in view of the intimate association between VEGF and tissue factor, enhanced growth factors could be a crucial driving force behind the hypercoagulable state. Notably, tissue factor acts as a cofactor to factor VIIa and is widely regarded as the physiological trigger to thrombin formation.[142]

Why are factors such as the angiopoietins involved? Angiopoeitin 1 and 2 are natural co-antagonists and both compete for the same binding site on Tie-2, an endothelial tyrosine kinase receptor. With an excess of angiopoeitin 1, stability of the endothelium is favoured, whereas the converse is true with an excess of angiopoeitin 2.[139] In these circumstances, the balance could ultimately favour endothelial destabilisation and therefore the action of cytokines such as VEGF.

Extracellular matrix turnover

The extracellular matrix is a dynamic structure, which continually undergoes a process of structural remodelling.[143] Structural remodelling of the atria has already been discussed, and this process could contribute to the hypercoagulable state, by virtue of both enhanced blood stasis and an abnormal endocardium. Impaired matrix degradation in atrial fibrillation is well documented.[16–22,144] These abnormal changes could,

therefore, be important in atrial remodelling and therefore indirectly contribute to thrombogenesis. Perhaps more importantly, MMPs could be directly implicated in thrombogenesis by virtue of several known interactions with the coagulation cascade, most notably with plasmin.[145]

The first matrix proteins studied were MMP-1 and TIMP-1 in patients with non-valvular atrial fibrillation, not receiving anticoagulation.[17] This study demonstrated evidence of impaired matrix degradation in patients with atrial fibrillation, but this finding was not independently associated with the presence of atrial fibrillation on multivariate analysis. However, a significant correlation was seen between the MMP/TIMP system and echocardiographic measures of left-ventricular hypertrophy and ventricular remodelling, but with no relation to atrial dimension or function. Notably, an independent relation was also shown between the MMP/TIMP system and the prothrombotic state, as assessed by prothrombin fragments 1 and 2. Similarly enhanced MMP-2 and MMP-9 are also associated with reduced PAI-1 activity, offering further links with thrombogenesis.[146] Other MMPs have also been investigated—eg, upregulation of myocardial MMP-9 and TIMP-3 shown in the left atrium of explanted hearts from patients with atrial fibrillation undergoing heart transplantation.[22] Additionally, MMP-14 concentrations in the right atrium were reduced.[22] These results should be interpreted with caution since all patients had advanced heart failure; however, atrial fibrillation could be associated with chamber-specific alterations in myocardial collagen content and MMP and TIMP amounts, indicative of differential remodelling and altered collagen metabolism.

Nitric oxide

Nitric oxide is synthesised by nitric oxide synthase, which is present in large concentrations in the endothelium. The expression of nitric oxide synthase is regulated by flow-mediated shear stress and is consequently downregulated at sites with low flow velocity.[147] Nitric oxide shows potent antithrombotic effects in arterial endothelium, and nitric oxide released from activated platelets inhibits platelet recruitment to the growing thrombus,[148] while also inhibiting expression of PAI-1.[149]

In animal models of atrial fibrillation, the loss of atrial contraction and consequent reduction in shear stress seems to reduce LA expression of nitric oxide synthase with a corresponding decrease in nitric oxide bioavailability and increase in PAI-1 expression.[150] In the LAA, nitric oxide concentrations were also significantly reduced compared with control animals, but this finding did not indicate decreased expression of nitric oxide synthase at this site. Since atrial thrombus is frequently formed in the LAA, this finding still has no adequate explanation.

	Relevance to thrombogenesis
Statins	Does statin use prevent atrial fibrillation—eg, by reducing inflammation?
Steroids in cardiac surgery	Postoperative atrial fibrillation is common with high rate of stroke; do steroids result in less inflammation and lower risk of stroke?
Blood and LAA markers of oxidative stress	Oxidative stress could drive endothelial damage and have procoagulant activity
Atrial fibrillation and endothelial dysfunction by flow-mediated dilatation	Endothelial dysfunction is well described in atrial fibrillation; measurement of flow-mediated dilatation relies on presence of sinus rhythm
New antithrombotic drugs (eg, oral factor Xa inhibitors, oral direct thrombin inhibitors)	Changes in indices of thrombogenesis with respect to different doses or drug regimens

LAA=left atrial appendage.

Table: **Ongoing clinical study areas on atrial fibrillation relevant to thrombogenesis**

Renin-angiotensin-aldosterone system (RAAS)

The RAAS is now appreciated as key to the pathophysiology of various cardiovascular disease states. The extent of these changes seems to relate predominantly to the reduction in angiotensin-II amounts. Atrial tissue has the capacity to produce and use this hormone with local expression of acetylcholinesterase and angiotensin-II receptors, both of which could be upregulated in atrial fibrillation.[151] RAAS could be mechanistically implicated in initiation and perpetuation of atrial fibrillation,[151–153] as well as providing the link to other mechanisms promoting the prothrombotic state in atrial fibrillation.

Angiotensin II has been shown to possess several proinflammatory properties and increases the production of proinflammatory cytokines (eg, interleukin 6 and tumour necrosis factor α [TNFα]), adhesion molecules (eg, vascular-cell adhesion molecule 1), monocyte chemoattractant protein 1, and selectins (eg, P-selectin).[154–156] Similarly, through release of various chemokines (eg, cytokine-induced neutrophil chemoattractant), angiotensin II can initiate neutrophil recruitment.[156] Expression of angiotensin-II receptors has also been linked with increased atrial cell death and leucocyte infiltration.[157] These data potentially support a complex relation between RAAS, inflammation, and atrial fibrillation.

Additionally, RAAS has been implicated in the activation of various MMPs and thromboxane A_2 (a prothrombotic signalling molecule produced by activated platelets). These processes could occur both as a direct effect of angiotensin II and also through induction of interleukin 6.[158] Furthermore, angiotensin II could accelerate degradation of nitric oxide through production of reactive oxygen species and thereby impair endothelium dependent vasodilatation.[159] Likewise, activation of RAAS increases synthesis of PAI-1, possibly indicating either enhanced endothelial damage or impaired fibrinolysis in atrial fibrillation.[160]

Unsurprisingly, modulation of the RAAS cascade has beneficial clinical outcomes.[152,153] A substudy of the Losartan Intervention For Endpoint reduction in hypertenstion (LIFE) trial assessed a cohort of patients with atrial

fibrillation and ECG left-ventricular hypertrophy assigned to either losartan or atenolol.[161] The rate of cardiovascular morbidity, mortality, and stroke was significantly reduced in patients receiving losartan despite similar reductions in blood pressure between the two trial groups. Thus, RAAS modulatory drugs are often considered in patients with atrial fibrillation for both stroke reduction and rhythm suppression.

Future directions

There is increasingly strong evidence for the presence of a prothrombotic or hypercoagulable state in atrial fibrillation. The presence of various flow and structural defects has been used to refine clinical risk stratification models for stroke and thromboembolism, or to help predict the likelihood of success for cardioversion and the long-term maintenance of sinus rhythm.[162]

However, the clinical role of indices of the prothrombotic or hypercoagulable state is emerging, although more data are clearly needed. For example, plasma vWf[82] and D-dimer[72,163] have been used to refine clinical stroke risk stratification. The availability of these biomarkers would be of particular value in patients classed as moderate risk, in whom clinical guidelines state that the use of aspirin or warfarin is possible, but measurement of high vWf amounts, for example, could reclassifiy such patients as high risk.[82] Also, these biomarkers could serve as indices of ongoing thrombogenesis, to test antithrombotic regimens (eg, warfarin plus an antiplatelet drug)[69] or new antithrombotic drugs (eg, oral thrombin inhibitors, oral factor Xa inhibitors), and help decision making on dose selection.[164] Application of such surrogate markers to test antithrombotic regimens has been evident in studies,[55,103] which have suggested that oral anticoagulation would be better than aspirin-clopidogrel combination therapy in reducing thrombogenesis in atrial fibrillation, a finding later confirmed in clinical trials showing the effectiveness of warfarin for stroke prevention.[104] The potential of such an approach was recognised in recommendations from a consensus conference organised by the German Atrial Fibrillation Competence NETwork and the European Heart Rhythm Association on defining outcome parameters for trials in atrial fibrillation.[165] The table summarises ongoing studies addressing areas of clinical interest with some relevance to thrombogenesis in atrial fibrillation.

Conclusion

The mechanisms underlying thrombogenesis in atrial fibrillation are clearly complex and remain only partly understood. Abnormal changes in flow, vessel wall, and blood constituents in atrial fibrillation fulfil Virchow's triad for thrombogenesis, and accord with a prothrombotic or hypercoagulable state in this arrhythmia. That this process is related purely to blood stasis is no longer accepted. Various abnormal changes related both to atrial fibrillation and its comorbidities impart a synergistic effect in maintaining a hypercoagulable state in this condition.

Conflict of interest statement

GL has received funding for research, educational symposia, consultancy, and lecturing from different manufacturers of drugs used for the treatment of atrial fibrillation and thrombosis. He was clinical adviser to the Guideline Development Group writing the UK National Institute for Health and Clinical Excellence (NICE) Guidelines on atrial fibrillation management, and coauthor of the 8th American College of Chest Physicians guidelines on antithrombotic therapy for atrial fibrillation. TW and ES declare that they have no conflict of interest.

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