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INVITED REVIEW

The prothrombotic state in atrial fibrillation: pathophysiological and management implications

Ahsan A. Khan[1] and Gregory Y.H. Lip[1,2,3]*

[1]Institute of Cardiovascular Sciences, University of Birmingham, City Hospital, Dudley Road, Birmingham, B18 7QH, UK; [2]Liverpool Centre for Cardiovascular Science, University of Liverpool and Liverpool Heart & Chest Hospital, Liverpool, UK; and [3]Aalborg Thrombosis Research Unit, Department of Clinical Medicine, Faculty of Health, Aalborg University, Aalborg, Denmark

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Abstract Atrial fibrillation (AF) is the commonest sustained cardiac arrhythmia and is associated with significant morbidity and mortality. There is plenty of evidence available to support the presence of a prothrombotic or hypercoagulable state in AF, but the contributory factors are multifactorial and cannot simply be explained by blood stasis. Abnormal changes in atrial wall (anatomical and structural, as 'vessel wall abnormalities'), the presence of spontaneous echo contrast to signify abnormal changes in flow and stasis ('flow abnormalities'), and abnormal changes in coagulation, platelet, and other pathophysiologic pathways ('abnormalities of blood constituents') are well documented in AF. The presence of these components therefore fulfils Virchow's triad for thrombogenesis. In this review, we present an overview of the established and professed pathophysiological mechanisms for thrombogenesis in AF and its management implications.

Keywords Atrial fibrillation • Prothrombotic state • Hypercoagulable • Thromboembolism

1. Introduction

Atrial fibrillation (AF) is an independent risk factor for thromboembolism and stroke, leading to an approximate three- to five-fold excess risk, and the prevention of thromboembolism is the cornerstone of AF management.[1] In addition to the haemodynamic changes associated with this arrhythmia, there are multiple causes to explain the unfavourable thromboembolic effects of AF. These include malfunctioning of the clotting cascade, inappropriate/excessive platelet activation, abnormal haemostasis process along with aberrant blood stasis, and presence of structural heart disease.[2]

The predisposition to thrombus formation (thrombogenesis) in AF can be described in relation to Virchow's triad for thrombus formation, such as abnormal blood flow, abnormal vessel structure, and abnormal blood constituents.[2] While Virchow originally described the abnormalities in relation to venous thrombosis, the triad of abnormalities can be applied to AF, as follows: (i) *Abnormal blood flow* in AF is evident within dilated cardiac chambers and the association with heart failure, with stasis and reduced left atrial appendage (LAA) velocities, and the presence of spontaneous echo contrast (SEC); (ii) *Abnormal vessel wall* is recognized as endothelial or endocardial damage or dysfunction (and related structural abnormal changes), with an increased risk of thromboembolism with structural heart disease; and (iii) *Abnormal blood constituents* with abnormalities of coagulation, fibrinolysis and platelets.[2,3]

By such fulfilment of Virchow's triad for thrombogenesis, AF therefore leads to a hypercoagulable state, a concept that was first proposed in 1995, with the associated implications for clinical practice.[2,4] In this review article, we present an overview of the established and professed pathophysiological mechanisms for thrombogenesis in AF and its management implications.

2. Anatomical and structural considerations

2.1 Atrial structure and function: the LAA and atrial wall

Each atrium is attached to a hollow structure known as an appendage. The LAA is the remnant of the embryonic left atrium, while the smooth-walled left atrium is derived from the primaeval pulmonary vein and its branches. The LAA is largely considered to be non-functional. However, pulse Doppler echocardiography has shown LAA to be an actively contracting structure with its prominent muscular trabeculations.[5]

The LAA is considered a key site for thrombus formation in patients with AF.[6] It has a narrow inlet and is long, thus providing ideal conditions for blood stasis, so unsurprisingly the LAA is the major site of intra-atrial thrombus formation even in patients with sinus rhythm.[7,8] In a transoesophageal echocardiogram (TOE) study which included 137 patients with AF and a recent embolic event, about one-fifth had evidence of thrombus in the LAA.[9] Another pooled analysis of 23 studies looking at 4792 patients with AF who underwent TOE, cardiac surgery or autopsy

* Corresponding author. Tel: +44 151 794 9020, E-mail: gregory.lip@liverpool.ac.uk

showed that 14% had left atrial (LA) thrombi with majority originating in LAA.[7]

Late gadolinium enhancement cardiac magnetic resonance (CMR) imaging is a useful tool to detect LAA structural dysfunction. For example, Akoum *et al.*[10] investigated 178 patients with AF and showed that LAA fibrosis on CMR imaging was associated with reduced flow velocities in LAA, suggesting that such fibrotic changes of the LAA appendage can be associated with blood stasis, subsequent thrombus formation, and eventually increased stroke risk.

At the structural level, Goldsmith *et al.*[11] have described severe changes on the endocardium within the LAA compared with the right atrial appendage (RAA) on scanning electron microscopy, especially in AF when compared with sinus rhythm. In another study, Masawa *et al.*[12] have described LAA as a 'rough endocardium' macroscopically with a crumpled facade and noted several little areas of erosion of the endothelium and clusters of clot within LAA in patients with AF and cerebral embolism.

Frustaci *et al.*[13] performed endomyocardial biopsies and established abnormal atrial histology, which was present uniformly in patients with AF: myocarditis was evident in 66% (active in 25%), non-inflammatory focal cardiomyopathy in 17% and patchy fibrosis in 17%. Of note, these changes were all found in atria and not in ventricles. These findings were further confirmed by Boldt *et al.*[14] who also demonstrated fibrosis related changes in LA tissue in the setting of AF.

Echocardiography is a useful non-invasive tool to assess LAA function, morphology, and orifice area and some of these measurements are found to be directly correlated with thromboembolism risks (see *Table 1*). Changes in the morphology of the LAA and the left atrium occur as a result of AF, which can be correlated to subsequent thromboembolism.[3]

Several clinical trials have assessed the benefit of closing LAA in reducing stroke risk in patients with AF but with mixed results thus far.[33,34] The LAA is also a source of AF development, with arrhythmias initiating from LAA, as oppose to the more conventional location of pulmonary veins.[35]

2.2 Other structural considerations

Changes in the left ventricle can also affect LAA. For example, Doukky *et al.*[36] conducted a retrospective cohort study of 297 patients with AF who underwent TOE to evaluate for LA appendage thrombus (LAAT) and also found that B-type natriuretic peptide (BNP, indicating left ventricular wall stress) was an independent predictor of LAAT in AF.

3. Abnormal blood stasis

AF leads to inefficient atrial systolic function which leads to increased blood stasis; this effect is more evident in the elderly, where atrial 'kick' may account for a third of the stroke volume.[37] Prolonged duration of AF promotes increasing LA dilatation thus enhancing the environment for stasis.[38] LA enlargement is an independent risk factor for stroke in the general population and LA dilatation is further enhanced in the presence of mitral stenosis, leading to further stasis, and a propensity to thrombosis.[39,40] Conversely, moderate-severe (non-rheumatic) mitral regurgitation with AF may be protective against stroke especially in the setting of LA enlargement.[41] Thus, describing patients with mitral valve disease and AF who have the highest probability of stroke has proven difficult.

Aberrant blood stasis in the left atrium and LAA can be visualized on TOE with low-pulse wave Doppler velocities or SEC during episodes of AF.[8,42–44] In contrast, a quadriphasic pattern of blood flow is seen in sinus rhythm, signifying minimum blood stasis.[45] The presence of SEC is independently predictive of an increased risk of thromboembolism.[46] It occurs when there is an increased interaction between fibrinogen and erythrocytes, and their relative concentrations. Since more fibrinogen is needed to induce the same effect with lower number of haematocrits.[47–49] As some patients with AF are intravascularly deplete during a low-cardiac output state, this discovery could explain the increased stroke rates observed in this patient population.[50]

Crucially, SEC can still be detected in patients who have reverted to sinus rhythm from AF.[51] The prothrombotic state in AF also associates with the extent of LAA impairment as well as TOE indices of stroke risk.[52,53] For example, prothrombin fragment F1 + 2, thrombin-antithrombin III complex, and fibrinopeptide A correlate to SEC seen on TOE.[54,55]

4. Abnormal blood constituents

The third component of Virchow's triad refers to alterations in the blood constitutions, which are evident in AF (see *Figure 2*). The major promoters of thrombogenesis are the various intravascular proteins of the clotting cascade and platelets. Abnormal changes in these promoters and other constituents, such as growth factors, inflammatory cytokines, and extracellular matrix are present in patients with AF.

4.1 Abnormal changes in coagulation factors

Abnormal changes in clotting factors in AF are extensively described (see *Table 2*).

Excessive fibrin turnover has been described in patients with AF irrespective of onset.[73,77,79,82,94–96] Abnormal levels of hypercoagulable indices, such as thrombin-antithrombin complexes (a protein compound of thrombin and antithrombin) and prothrombin fragment F1 + 2 (activation peptide released from prothrombin during thrombin formation) are significant in patients with stroke who have AF compared to sinus rhythm.[97]

A relationship between various prothrombotic markers, stasis, and intracardiac thrombus is well described.[70,98]

4.1.1 D-dimer

D-dimer, also known as a fibrin degradation product is a small protein fragment present in the circulation after a clot undergoes fibrinolysis. It is present in several disease states with systemic manifestations. Elevated levels of D-dimer are found in unwell patients with severe infection, trauma, or inflammatory disorders. D-dimer seems to play an important role in cardiovascular disease states such as AF. Several studies have shown that D-dimer on its own can predict the occurrence of LAA thrombi on TOE. This has led researchers to deduce that measuring D-dimer may help predict the absence of LAA thrombi.[70,98] For detection of the latter, Somloi *et al.*[99] have proposed that D-dimer measurements are comparable to utilization of a TOE-based strategy with a very good negative predictive value of 98%.

In chronic AF, D-dimer concentrations generally remain stable over time and thus appear to be a practical standard for evaluating the extent of hypercoagulability regardless of patients' age.[94] D-dimer along with clinical risk factors has also shown to predict ensuing thromboembolic events in AF patients, including those already on anticoagulation.[86,92]

Table 1 Echocardiographic features of LAA and risk of stroke[6]

LAA parameters	Author (ref)	Study	Findings
LAA fibrosis	Akoum and Marrouche[15]	178 AF patients	Fibrosis of LAA directly correlated with reduced LAA flow velocities.
LAA function	Uretsky *et al.*[16]	141 patients (48 AF and 93 sinus rhythm)	Mean LAA contraction velocity lower in patients with LAA thrombus, AF, or history of previous stroke/transient ischaemic attack (TIA). Thirty-three percent of patients with LAA flow velocity ≤11 cm/s were found to have LAA thrombus.
	Lee *et al.*[17]	218 AF patients	Lower LAA flow velocity in patients with stroke
	Goldman *et al.*[18]	*Post hoc* analysis from the Stroke Prevention in AF III (SPAF-III) trial that included 721 patients who underwent TOE	Low LAA flow velocity independently linked with thrombus formation and risk of embolism.
	Lee *et al.*[19]	360 AF patients	Incidence of ischaemic stroke increases in patients with reduced LAA flow velocity.
	Wai *et al.*[20]	103 consecutive patients who underwent TOE and TTE	TTE LAA emptying velocity (LAA E) predicted the presence of thrombus or spontaneous echo contrast (SEC) independent of AF. Optimal TTE LAA E cut-off was ≤31 cm/s in AF patients (80% sensitive, 79% specific).
	Sasaki *et al.*[21]	TTE and TOE on 120 patients within 7 days of onset of acute ischaemic stroke	LA peak systolic strain was significantly correlated with LAA emptying flow velocity and significantly decreased in patients with LAA dysfunction as defined by severe SEC and/or presence of thrombus.
	Kumagai *et al.*[22]	98 consecutive patients who had stroke or TIA and then underwent TOE	LAA slow flow velocity was associated with the presence of thrombus.
	Doukky *et al.*[23]	297 AF patients	Patients with left atrial appendage thrombus (LAAT) had abnormal diastology.
	Kimura *et al.*[24]	123 AF patients	Patients with LAAT were found to have lower LAA blood flow velocity.
LAA morphology	Di Biase *et al.*[25]	932 AF patients	Prevalence of ischaemic stroke varied among different LAA morphologies, such as cauliflower shape (18%), cactus (12%), windsock (10%), and chicken wing (4%) (see *Figure 1*).
	Lee *et al.*[19]	360 AF patients	Chicken wing shape was associated with reduced stroke risk.
	Kimura *et al.*[26]	80 AF patients	Patients with cauliflower LAA morphology had high risk of stroke independent of anything else.
	Anselmino *et al.*[27]	348 AF patients undergoing elective ablation	Correlation between cerebral infarcts on MRI and non-chicken wing morphology.
	Khurram *et al.*[28]	1063 AF patients	Widespread LAA trabeculations linked with ischaemic stroke.
	Yamamoto *et al.*[29]	564 AF patients undergoing ablation	Number of LAA lobes independently correlated with presence of LAA thrombus.
LAA orifice area	Lee *et al.*[17]	218 AF patients	Larger LAA orifice area in patients with stroke.
	Lee *et al.*[19]	360 AF patients	Larger LAA orifice area associated with ischaemic stroke.
	SPAF Invesigators[30]	568 AF patients	LAA area >6 cm^2 associated with increased risk of arterial embolic events.
	Stollberger *et al.*[31]	409 AF patients	Increased LAA size was related with permanent AF. LAA size was not predictor for thromboembolic events.

Elevated D-dimer levels in patients on oral anticoagulation was also a predictor of cardiovascular adverse events.[92]

4.1.2 Von Willebrand factor

von Willebrand factor (vWf) is a large multimeric (containing two or more polypeptide chains) glycoprotein present in plasma and engineered constitutively as ultra-large vWf in endothelium, subendothelial connective tissue, and megakaryocytes (precursor to platelets). It is a key player in haemostasis. Measurement of plasma vWf levels have provided further insight into the hypercoagulable state in AF. vWf is a recognized marker of endothelial dysfunction, and increased vWf levels independently correlate with incidence of LAA thrombus in AF.[70] Additionally, elevated LAA endocardial expression of vWf is seen, particularly in patients with a bulky appendage; this appears to be related to the presence of adherent platelet thrombus.[67] Moreover, enhanced expression of vWf in the endocardial tissue is found to be related to dilated LA size in mitral valve pathology and wide myocyte diameter.[78]

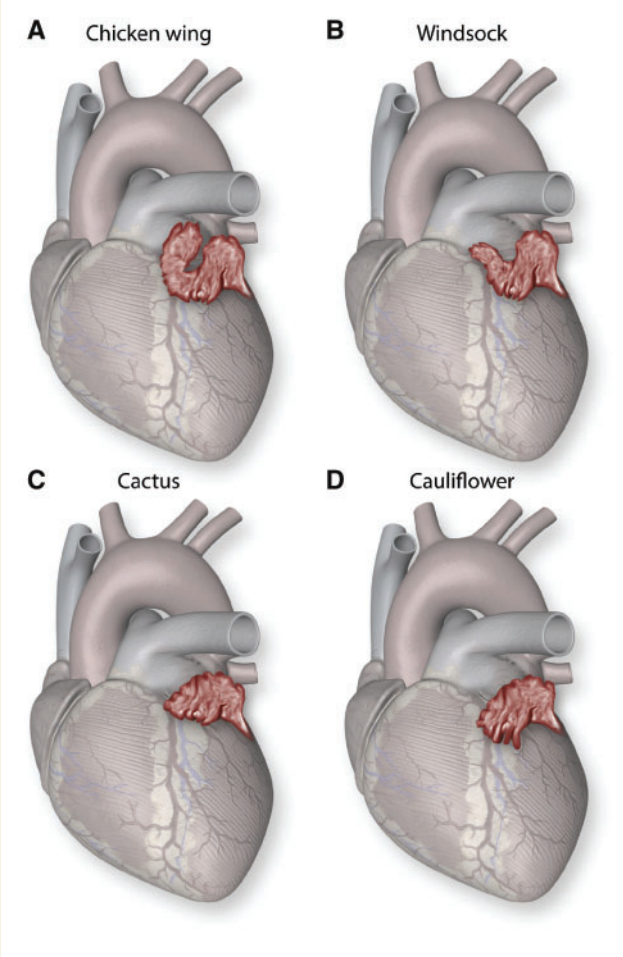

Figure 1 Diagram of the different morphologies of the left atrial appendage: (*A*) chicken wing, (*B*) windsock, (*C*) cactus, and (*D*) cauliflower. Adapted from Owais *et al.*[32]

Tissue factor, a co-factor to factor VIIa and widely regarded as the physiological trigger to thrombin formation and vWf are overexpressed in the atrial endocardial tissue in patients with AF with a previous record of cardiogenic thromboembolism.[84,100] Interestingly, patients receiving either aspirin or no antithrombotic treatment have shown positive association with plasma vWf and D-dimer levels. However, this is the not the case in patients receiving warfarin, thus confirming the capability of warfarin to alter the thrombogenic process.[79]

A positive association between AF and plasma vWf was also reported in the Rotterdam study.[101] This was most evident in females, and perhaps can explain the higher risk of stroke due to AF in females compared with males.[102] Plasma vWf levels were found to be elevated in patients with heart failure, previous stroke, diabetes, and those of older age. These are four independent risk factors for stroke and key components of the stroke risk stratification schema.[62,63] Moreover, further study of the data suggested that vWf concentrations may independently predict subsequent stroke and vascular events.[63,103] However, such clinical application will most likely be hampered by the poor specificity of vWf (and other similar biomarkers) since concentrations are also increased in multiple other disorders.[104,105]

4.1.3 Thrombin

Thrombin is usually seen as the end-product of the coagulation cascade and is thus responsible for the conversion of soluble fibrinogen into the insoluble fibrin clot. The coagulation pathway has several feedback loops and inhibitory circuits that are all directly or indirectly affected by thrombin concentration. Current techniques to evaluate haemostasis in clinical laboratories have their limitations, particularly in their sensitivity to detect hypercoagulable and mild hypocoagulable states.[106]

A different technique such as thrombin generation assay (TGA) may well be able to bridge this gap. Although present since 1950s, it has only recently become commercially available.[107] This evaluates thrombin generation (resulting from the action of procoagulant driver) and decay (as a result of the action of the anticoagulant driver) simultaneously and thus represent more accurately what happens *in vivo*.[107] Preliminary data suggests that TGA is affected by all anticoagulant drugs, and therefore, has the potential to be the prime assay to assess efficacy of these drugs, particularly in those individuals who bleed or have recurrent thrombosis despite being on a fixed dose of an anticoagulant.[107] TGA still lacks validation in clinical setting despite having several studies conducted.108–110 Given the large inter-laboratory variability of this method, it is still far away for being utilized clinically. Standardization and validation of this method are essential for its introduction into the clinical practice.

4.2 Abnormal fibrinolysis

Enhanced fibrinolysis as evidenced by excess levels of tissue-plasminogen activator (t-PA) antigen, plasminogen activator inhibitor-1 (PAI-1), and low amounts of plasmin-antiplasmin (PAP) complex can be directly correlated to a pathophysiological response to the hypercoagulable state.[75,111] t-PA is an enzyme found on endothelial cells and is involved in the lysis of blood clots by catalysing the conversion of plasminogen to plasmin and thus plays an important role in fibrinolysis. Conversely, PAI-1 acts as an antagonist to t-PA, preventing fibrinolysis. Understandably, elevated levels of PAI-1 act as a risk factor for thrombosis leading to a prothrombotic state.

Plasmin is a key enzyme in fibrinolysis as it is responsible for degradation of several plasma proteins including fibrin clots (see *Figure 3*). Once converted from the inactivated plasminogen, plasmin binds to the clot and its cleavage produces angiostatin, an endogenous angiogenesis inhibitor (blocks the growth of new blood vessels), this probably causes inhibition of cell migration, proliferation, and induction of apoptosis within the clot. Thus, plasmin's deficiency can contribute to the hypercoagulable state in vivo. Conversely, alpha-2-antiplasmin (α-2-plasmin) is an enzyme responsible for inactivating plasmin and in a normal physiological state prevents excessive bleeding.

PAP complex, a compound formed by reaction between plasmin and α-2-plasmin is also an important index of recent fibrinolytic activity. Increased PAP complex formation is associated with enhanced fibrin formation and an increased reactive plasminaemia. Increased levels of plasmin-anti-plasmin complexes were found to be independently linked to thromboembolic risk factors, such as older age (>75 years), recent congestive heart failure, decreased fractional shortening of the left ventricle and recent onset of AF in the Stroke Prevention in Atrial Fibrillation (SPAF) III study.[112] There is also a strong relationship between t-PA concentrations and LA diameter in AF.[95] Anticoagulation leads to improvement in fibrinolytic markers in rheumatic AF.[113]

High concentrations of t-PA and PAI-1 can also signify the presence of confounder comorbidities, such as ischaemic heart disease, heart failure

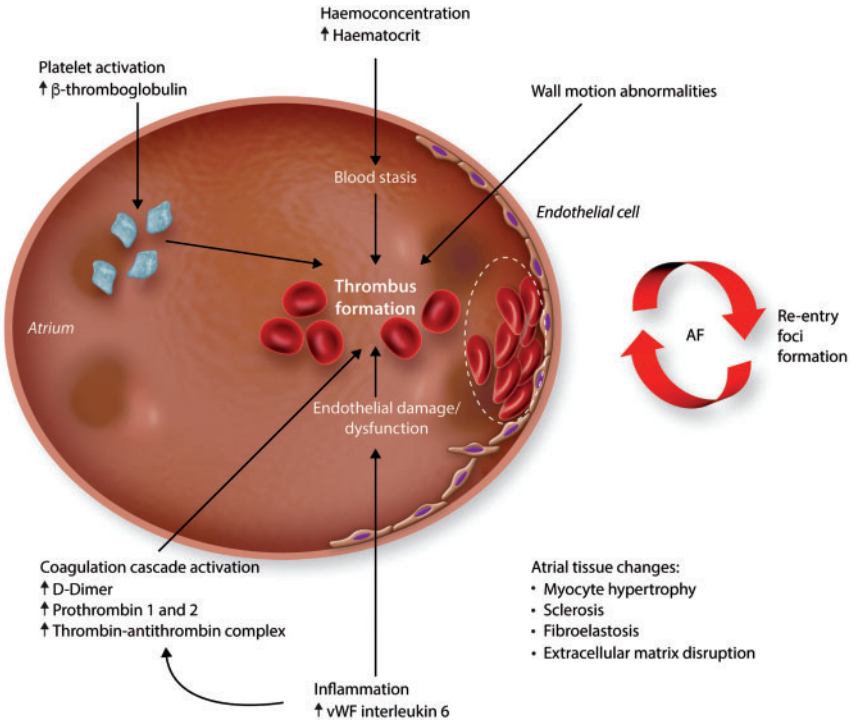

Figure 2 The prothrombotic state in AF—elements of coagulation system and their interaction with atrial tissue. Adapted from Watson *et al.*[3]

and hypertension, all of which can lead to endothelial dysfunction, damage, and inflammation. Nevertheless, the presence of AF *per se* independently modulates these biomarkers.[95,111,113] Consequently, high levels of t-PA and PAI-1 in AF could be as a result of endothelial damage or systemic inflammation.[114,115] PAI-1 concentrations are independent predictors of development of AF post-cardiopulmonary bypass as well as successful cardioversion.[116,117]

It is uncertain whether elevated levels of t-PA or PAI-1 in AF are as a result of endothelial damage, inflammation, fibrinolysis, vascular disease, or a combination. Conversely, abnormal changes in the fibrinolytic system might relate to thrombogenesis and structural remodelling of the atria, in view of the strong association to extracellular matrix turnover.[3]

4.3 Platelets

Numerous studies have shown a potential role for platelets in the prothrombotic state in AF but the results have been contradictory, indicative of the diverse aspects of platelet physiology that have been evaluated and possibly confounding from assay variability. Current evidence does support the concept that abnormal changes within platelets in AF do exist, but their correlation to increased thrombotic risk remains unclear. Such changes could simply indicate underlying vascular comorbidities.

Choudhury *et al.*[118] reported that patients with AF had increased levels of soluble P-selectin and platelet microparticles compared with healthy controls in sinus rhythm. However, no difference was observed compared with disease-matched controls, therefore, suggesting that these elevated levels are probably a consequence of the underlying comorbidities rather than AF itself. Enhanced β-thromboglobulin, a platelet-specific protein that is released from α-granules during platelet aggregation and subsequent thrombus formation and indicates platelet activation, has been found in patients with both valvular and non-valvular AF compared with controls in sinus rhythm.[50,53,80,119–122]

Anticoagulation therapy modulates only some of these abnormal platelet changes. For example, Kamath *et al.*[74] were unable to show a beneficial effect of anticoagulation on plasma β-thromboglobulin concentrations. Furthermore, *in vitro* measures of platelet aggregation were not significantly elevated in AF, thus probing the value of platelets in augmenting the prothrombogenic drive in this setting. Additionally, oral anticoagulation does not decrease platelet activation in AF, in spite of marked inhibition of other coagulation variables.[123,124] Conversely, aspirin reduces levels of soluble P-selectin when compared with warfarin in AF,[125] but some individuals given high-dose aspirin (325 mg per day) still did not demonstrate inclusive inhibition of platelet aggregation.[126]

While dual antiplatelet treatment is more effective in inhibiting platelet function than monotherapy alone, this approach does not considerably affect the markers of the coagulation cascade, such as antithrombin III, thrombin-antithrombin III complex, platelet-dependent thrombin generation, prothrombin fragment F1 + 2 in patients with AF.[127] This is consistent with clinical trials that have now shown that dual antiplatelet treatment with aspirin and clopidogrel was less efficacious than warfarin for stroke prevention in AF.[128] These clinical findings are also corroborated by data demonstrating that patients show alteration in plasma markers of platelet function but not platelet aggregation, which are impervious to anticoagulation.[74]

Sohara *et al.*[129,130] have suggested that improvement of platelet activity and coagulability occur within the first 12 h of developing AF and a

Table 2 Examples of abnormal changes in coagulation in AF

Author	Study	Findings
Alonso *et al.*[56]	1209 AF	↑Fibrinogen (HR 1.13), ↑vWF (HR 1.17), and ↑factor VIIIc (HR 1.17); increased levels associated with poor outcomes.
Ancedy *et al.*[57]	122 AF	↑vWF
Asakura *et al.*[58]	83 AF vs. healthy controls	↑TATIII and PF1 + 2
Christersson *et al.*[59]	18 201 AF	↑D-dimer levels associated with increased incidence of stroke or systemic embolism, death, and major bleeding.
Chung *et al.*[60]	25 AF vs. 35 coronary artery disease (CAD) vs. 30 healthy controls	↑Tissue factor (TF), ↑vascular endothelial growth factors (VEGF) in patients with AF, and CAD compared with controls.
Conway *et al.*[61]	37 AF	↑CRP, soluble P-selectin, and haematocrit
Conway *et al.*[62]	1321 AF	↑vWF
Conway *et al.*[63]	994 AF	vWF is not a significant predictor of stroke and vascular events
Feinberg *et al.* (SPAF III)[64]	1531 AF	PF1 + 2 not linked with thromboembolism
Freestone *et al.*[65]	59 AF; 40 healthy controls	↑vWF
Fu *et al.*[66]	90 AF	↑soluble P-selectin; ↑fibrinogen
Fukuchi *et al.*[67]	AF vs. without AF	↑vWF in atrial appendage tissue
Gustafsson *et al.*[68]	20 AF with stroke; 20 AF without stroke; 20 stroke without AF; 40 healthy controls	↑vWF and D dimer in patients with AF
Hatzinikolaou-Kotsakou *et al.*[69]	18 PAF; 17 persistent AF patients who underwent successful elective cardioversion; 20 permanent AF	Permanent AF associated with ↑vWF, fibrinogen levels and soluble P-selectin ($P < 0.001$). Persistent AF associated with ↑vWF ($P = 0.0064$) and fibrinogen levels ($P = 0.002$) but not P-selectin ($P = 0.509$). PAF associated with ↑fibrinogen ($P = 0.003$) and P-selectin ($P = 0.005$) but not vWF ($P = 0.61$)
Heppell *et al.*[70]	109 AF with or without thrombus in left atrium	↑vWF, D-dimer, and TATIII in patients with LA thrombus
Hu *et al.*[71]	894 AF	↑GDF-15
Inoue *et al.*[72]	246 AF; 111 healthy controls	↑D-dimer in AF with risk factors but not significant in PF1 + 2
Kahn *et al.*[73]	75 AF vs. 42 controls	vWF higher in AF after stroke
Kamath *et al.*[74]	93 AF; 50 healthy controls	↑D-dimer
Kamath *et al.*[75]	31 acute onset AF; 93 permanent AF; 31 healthy controls	Haematocrit raised in acute AF whereas ↑D-dimer in permanent AF but not in acute AF
Kanda *et al.*[76]	34 AF vs. 14 controls	↑Fibrinopeptide A (FPA) in the AF group compared with control ($P < 0.05$)
Kumagai *et al.*[77]	73 AF; 73 controls	↑D-dimer
Kumagai *et al.*[78]	16 AF post-mortem	↑vWF, mRNA and protein in enlarged atriums
Lip *et al.*[79]	87 AF; 158 controls	↑vWF and D-dimer in AF patients
Lip *et al.*[80]	51 AF; 26 healthy controls	↑D-dimer
Lopez-Castaneda *et al.*[81]	107 AF	↑vWF, ↑high-molecular-weight multimers; lower ADAMTS13
Marin *et al.*[82]	24 acute onset AF; 24 chronic AF vs. 24 coronary artery disease in sinus rhythm; 24 healthy controls	↑vWF, D-dimer, and s-thrombomodulin in all AF groups with no significant difference post-cardioversion
Mondillo *et al.*[83]	45 AF; 35 healthy controls	↑vWF, D-dimer, and s-thrombomodulin
Nakamura *et al.*[84]	LAA tissue samples of seven AF vs. four without AF	↑TF expression and vWF
Nozawa *et al.*[85]	509 AF; 111 healthy controls	↑D-dimer, non-significant in PF1 + 2
Nozawa *et al.*[86]	509 AF	↑D-dimer but not PF1 + 2 with predictive significance for thromboembolic events
Ohara *et al.*[87]	591 AF; 129 controls	↑D-dimer, PF1 + 2, platelet factor 4, and β-thromboglobulin in AF; D-dimer and prothrombin fragment F1 + 2 predict stroke risk
Roldan *et al.*[88]	829 AF	vWF found to be an independent risk factor for unfavourable events.
Sakurai *et al.*[89]	28 atrial flutter; 27 controls	↑D-dimer in patients with poor LAA function
Shinohara *et al.*[53]	45 AF	↑TATIII and D dimer in patients with low-LAA velocity vs. patients with high-LAA velocity
Siegbahn *et al.*[90]	6202 AF	↑D-dimer; baseline D-dimer results were related to the rate of stroke/systemic embolism
Sohara and Miyahara[91]	13 paroxysmal AF vs. healthy controls	Non-significant in D-dimer and TATIII

Continued

Table 2 *Continued*

Author	Study	Findings
Vene *et al.*[92]	113 AF	↑D-dimer in AF with cardiovascular events vs. no events
Wu *et al.*[93]	5412 AF vs. 29 292 controls	↑Circulating haemostatic markers (PF-4, BTG, P-selectin, D-dimer, Fibrinogen, TAT, F1 + 2, AT-III, and vWf) in patients with AF

ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; AT-III, antithrombin III; BTG, B-thromboglobulin; GDF, growth differentiation factor; LA, left atrium; LAA, left atrial appendage; mRNA, messenger RNA; PF1 + 2, prothrombin fragment F1 + 2; PF4, platelet factor 4; s-thrombomodulin, soluble thrombomodulin; TATIII, thrombin-antithrombin III complex; TF, tissue factor; vWF, von Willebrand factor.

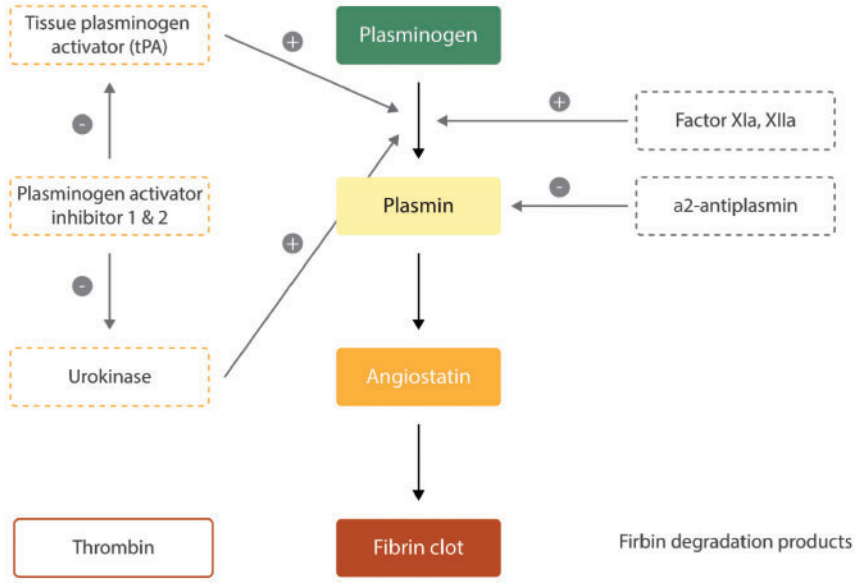

Figure 3 Schematic representation of fibrinolysis process including key components.

substantial reduction in platelet activity is seen after restoration of sinus rhythm compared with controls at 24 h post-cardioversion. This is consistent with the study by Akar *et al.*[131] showing that AF causes local cardiac platelet activation within minutes of onset.

Despite the presence of enhanced platelet activation in AF, there is inadequate data to prove that it directly augments thrombotic risk. A substudy from SPAF-III trial showed no correlation between plasma β-thromboglobulin levels and later thromboembolic events.[64] On the contrary, the population-based Rotterdam study reported that plasma soluble P-selectin concentrations were extrapolative of unfavourable clinical outcomes in elderly patients with AF.[132]

In view of the strong association between platelet activation and the atherothrombotic vascular comorbidities related to AF, the platelet activation observed in AF could contribute to thrombogenesis indirectly. For example, high expression of P-selectin on platelets coupled with reduced concentrations of nitric oxide has also demonstrated to be a risk factor for silent cerebral infarction in patients with AF.[133] Additionally, elevated P-selectin and CD63 levels have both been linked with the preembolic and embolic status of patients with non-rheumatic AF.[134]

The excess thromboembolic risk in AF is more related to the activation of the coagulation cascade as oppose to platelets, since platelet activation in AF is no more than what would be expected due to concurrent comorbidities.[74,80,118] Warfarin reduces greater number of plasma coagulation factor-related prothrombotic indices compared to platelet related indices.[74,80] This is consistent with data that warfarin (as a modulator of the coagulation cascade) is more efficacious than aspirin (a platelet inhibitor) as thromboprophylaxis in AF.

Anticoagulation therapy has demonstrated reduced concentrations of several prothrombotic markers.[79,135,136] This still applies with low-intensity anticoagulation (International Normalized Ratio (INR) 1.5–1.9), where D-dimer and prothrombin fragment F1 + 2 levels are suppressed.[85] Thus, in Phase II clinical trials of the non-vitamin K antagonist oral anticoagulants, markers of hypercoagulability have been utilized as surrogate indices of efficacy to assess the efficacy of antithrombotic treatments (e.g. AZD0837, Edoxaban, and Dabigatran).[137–139]

Recent developments in the understanding of haemostasis and thrombosis have identified new targets for development of new anticoagulants. Data from epidemiological studies and various animal models have provided an insight into the 'contact pathway' as a potential mediator of thrombosis that plays a negligible part in haemostasis.[140] Factor XI and XII have been recognized as potentially safer targets of anticoagulation than thrombin or FXa.[140] Further studies are clearly warranted to

explore this avenue and if successful could address the current unmet medical needs of safer anticoagulation with minimal risk of bleeding compared to existing anticoagulant agents.

4.3.1 Impact of restoration of sinus rhythm

Oltrona *et al.*[141] showed that plasma levels of the markers of thrombin generation and activity were significantly increased acutely in patients with AF who had pharmacological cardioversion to sinus rhythm. Coagulation system is more prominently activated following electrical cardioversion as compared to a pharmacological strategy.[142] The same study found that the energy delivered for cardioversion and plasma D-dimer values on Day 7 were positively correlated.[142] Perhaps unsurprisingly, a prolonged duration of AF could lead to a well-established prothrombotic state (as estimated by D-dimer value) post-cardioversion.[143] This prothrombotic state following cardioversion has been noticed despite optimum anticoagulation.[144] Unsurprisingly, patients receiving therapeutic low-molecular weight heparin prior to cardioversion appear to have less hypercoagulability.[145] According to limited data, in atrial flutter, plasma amounts of prothrombin fragment F1 + 2, platelet factor 4, D-dimer, thrombin-antithrombin III complex, and β-thromboglobulin stay elevated but do not seem to increase further following cardioversion.[89]

5. What promotes the prothrombotic state in AF?

Multiple mechanisms have been reported to 'drive' the prothrombotic or hypercoagulable state in AF but most evidence has focused on the potential role of inflammation and various growth factors.

5.1 Inflammation

In AF, inflammation might not only lead to endothelial damage, dysfunction, or activation but can also be directly attributed to thrombogenesis *per se*. Growing data have proposed an association between inflammation and the instigation and maintenance of AF.[146–150] Additionally, abnormal inflammatory markers have shown collaboration with hypercoagulable indices in AF, suggesting a crucial role of inflammation in the prothrombotic state in AF.[146]

Majority of AF cases are associated with diverse comorbidities, many of which can augment the inflammatory state; however, there is potentially a direct association between AF and inflammation. Interleukin-6 concentrations are found to be abnormal in patients with AF, with implications on prognosis.[151] Levels of high-sensitivity C-reactive protein (hs-CRP) are higher in patients with AF than sinus rhythm controls, with a gradual increase in hs-CRP seen with the transition from sinus rhythm to paroxysmal and subsequently persistent AF.[114] Elevated levels of hs-CRP consistently associate with cardiovascular risk, while not necessarily with future AF.[146] Conversely, low levels of hs-CRP and E-selectin at baseline are linked with a higher probability of sinus rhythm maintenance at 6 months following electrical cardioversion of AF.[152] Nevertheless, sinus rhythm preservation does not appear to have an effect on hs-CRP levels, suggesting a relationship to underlying comorbidities.[152] High-hs-CRP concentrations demonstrated predictive mortality and vascular death in AF but not stroke itself.[153]

What is clear is that inflammation is intimately linked to thrombogenesis in patients with AF. CRP and interleukin-6, both markers of inflammation invigorate tissue factor production from monocytes *in vitro*.[154,155] Interleukin-6 enhances platelet generation and sensitivity to thrombin, augments transcription of fibrinogen and is associated with both endothelial activation and damage.[156,157] Interleukin-6 is also independently associated with levels of tissue factor and increased stroke risk, while fibrinogen levels and plasma viscosity are independently linked to hs-CRP.[158]

5.2 Extracellular matrix turnover

The extracellular matrix is a dynamic structure and provides structural integrity for myocytes,[159] but continually undergoes a process of structural remodelling.[160] Impaired extracellular matrix degeneration in AF is well described, perhaps contributing to atrial remodelling, and therefore, indirectly leads to thrombogenesis.[161–168] More importantly, matrix metalloproteinases (MMPs) by virtue of several known interactions with the coagulation cascade such as plasmin could be directly implicated in thrombogenesis.[169] MMPs are calcium-dependent, zinc-containing enzymes belonging to a large family of enzymes known as metzincin superfamily. Collectively, they are responsible for degrading various types of extracellular matrix proteins.

The first matrix proteins considered were MMP-1 and Tissue inhibitor of metalloproteinase (TIMP)-1 in patients with AF not receiving anticoagulation by Marín *et al.*[162] Their work showed confirmation of defective matrix degradation in patients with AF. However, this finding was not independently related to the presence of AF on multivariate analysis.[162] Remarkably, an independent correlation was additionally seen between the MMP/TIMP system and the hypercoagulable state, as measured by prothrombin fragment F1 + 2.

Equally, improved MMP-2 and MMP-9 have been linked with low PAI-1 activity, providing further connection with thrombogenesis.[170] AF could also be allied with chamber-specific adaptations in myocardial collagen content along with MMP and TIMP amounts, suggestive of incongruous remodelling and modified collagen metabolism.[167]

5.3 Nitric oxide

Nitric oxide is synthesized by nitric oxide synthase which is available readily in the endothelium. Flow-mediated shear stress regulates the expression of nitric oxide synthase, and is therefore, down-regulated at sites with low flow velocity.[171] In arterial endothelium, nitric oxide has demonstrated strong antithrombotic effects, when released from activated platelets.[172] Nitric oxide restrains platelet deployment to the developing thrombus, while also impeding activity of PAI-1.[173]

Animal models of AF have displayed low nitric oxide bioavailability and an increase in PAI-1 expression due to diminished LA expression of nitric oxide synthase as a result of impaired atrial contraction and a subsequent decrease in shear stress.[174] Nitric oxide concentrations are also reduced in the LAA compared with control animals, providing further evidence to the notion that atrial thrombus is a common occurrence in the LAA.

5.4 Renin-angiotensin-aldosterone system

The Renin-angiotensin-aldosterone system (RAAS) is widely recognized as vital to the pathophysiology of various cardiovascular conditions, with the most important component being angiotensin II. The endothelium in atria has the capacity to produce and utilize this hormone due to acetylcholinesterase and angiotensin II receptors being present, both of which could be up-regulated in AF.[175] It is conceived that RAAS has a key role in instigation and perpetuation of AF along with providing support to other elements promoting the hypercoagulable state in AF.[175–177]

Table 3 Different antithrombotic agents and their action on prothrombotic factors

Class	Agent	Prothrombotic factor	Effect
Vitamin K antagonist	Warfarin	Factor II, VII, IX, X	Reduces levels[186]
		D-dimer	Reduces levels[79,80,85,96,139]
		Beta-thromboglobulin	Reduces levels[80]
		Prothrombin fragment F1 + 2	Reduces levels[85,136,187]
		Thrombin-antithrombin III complex (TAT)	Reduces levels[58,96]
		Tissue-plasminogen activator (t-PA)	Reduces levels[96]
	Acenocumarol	Tissue-plasminogen activator (t-PA)	Reduces levels[135]
		Plasminogen activator inhibitor-1 (PAI-1)	Reduces levels[135]
		Antithrombin III	Reduces levels[135]
		D-dimer	Reduces levels[135]
Non-vitamin K antagonist oral anticoagulants (NOACs)	Dabigatran	Thrombin	Reduces levels[188]
		D-dimer	Reduces levels[139]
	Rivaroxaban	Factor Xa	Reduces levels[189]
	Apixaban	Factor Xa	Reduces levels[190]
	Edoxaban	Factor Xa	Reduces levels[191]
	AZD0837	Thrombin	Reduces levels[137]
		D-dimer	Reduces levels[137]
Antiplatelets	Aspirin	Thromboxane A_2	Reduces platelets aggregation[192,193]
		Beta-thromboglobulin	No change[80]
		Platelet factor 4 (PF 4)	Reduces levels[194]
		Thrombin-antithrombin III complex (TAT)	Reduces levels[58]
		Prothrombin fragment F1 + 2	Reduces levels[58]
	Clopidogrel	Platelets	Reduces platelets aggregation[195]
	Ticagrelor	Platelets	Reduces platelets aggregation[196]
	Prasugrel	Platelets	Reduces platelets aggregation[197]

Angiotensin II has many pro-inflammatory properties and enhances the assembly of pro-inflammatory cytokines, such as interleukin-6 and tumour necrosis factor α; adhesion molecules e.g. vascular-cell adhesion molecule 1; monocyte chemo-attractant protein 1, and selectins, such as P-selectin.[178–180] Through the release of different chemokines, for example, cytokine-induced neutrophil recruitment, angiotensin II can initiate neutrophil recruitment.[180] The presence of angiotensin II receptors is strongly correlated with increased atrial cell death and leucocyte infiltration.[181] This suggests a complex relationship between RAAS, inflammation, and AF.

Furthermore, RAAS has been associated with the activation of thromboxane A_2, a prothrombotic signalling molecule produced by activated platelets and various MMPs. This process happens as a direct effect of angiotensin II and induction of interleukin-6.[182] Additionally, angiotensin II can weaken endothelial dependent vasodilation by accelerating degradation of nitric oxide through generation of reactive oxygen species.[183] Similarly, PAI-1 synthesis is increased through activation of RAAS suggesting either pronounced endothelial damage or defective fibrinolysis in AF.[184]

Thus, it perhaps comes as no surprise then that modification of the RAAS cascade has beneficial clinical outcomes.[176,177] A substudy of the Losartan Intervention For Endpoint reduction in hypertension (LIFE) trial of patients with AF and LVH on an electrocardiogram (ECG) found that the incidence of stroke, cardiovascular morbidity, and mortality was significantly less in patients receiving losartan as compared to atenolol despite achieving similar reductions in blood pressure.[185]

6. Management implications

Several studies have looked at the current range of antithrombotic treatment available and their effects on the various prothrombotic factors related to AF (see *Table 3*).

Indices related to the prothrombotic or hypercoagulable state in AF have been used as biomarkers ('biological markers') to improve clinical risk stratification models for stroke/thromboembolism or bleeding, and/or assist in predicting the likelihood of success for cardioversion and preservation of sinus rhythm. Such biomarker research in AF has contributed to our current understanding of the pathophysiological mechanisms involved both in the development of arrhythmia, its underlying substrate and its long-term complications.

Many biomarkers in AF have provided independent information on increased risk of different outcomes (see *Table 4*). However, many biomarkers are predictive of multiple adverse outcomes in AF, and in many studies the incremental predictive value over clinical factor based risk scores is marginal, although statistically significant. Of note, biomarkers are related to several other important outcomes in patients with AF, such as risk of dementia, congestive heart failure, and myocardial infarction.[198] The other issue is the laboratory variability and diurnal variation in some biomarkers, as well as costs and practicality. Given that such biomarkers are indicative of many comorbidities (hence, a 'sick' heart) and are non-specific, these tests may be better utilized as 'rule out' rather than 'rule in' for management decision-making.

Table 4 Schematic representation of the strength of association of different biomarkers and outcomes in patients with AF

Biomarker	Stroke/systemic embolism	Mortality	Major bleeding
Cardiac biomarkers			
Troponin	+++	+++	+++
NT-proBNP	+++	+++	+
Renal dysfunction	+/++	++	++
Inflammation biomarkers			
CRP	+	+++	+
IL-6	++	+++	++
GDF-15	++	+++	+++
Galectin-3	+	+++	+
Endothelial function			
vWF	+	++	++
Coagulation			
D-dimer	++	++	++

Adapted from Hijazi *et al.*[198]
+, outcomes in analysis unadjusted; ++, adjusted for clinical risk factors; +++, adjusted for clinical risk factors and other biomarkers.

Biomarkers were first proposed to refine clinical risk stratification in 2006, where high vWf levels are additive to the $CHADS_2$ and Birmingham (now CHA_2DS_2VASc) clinical scores to refine risk prediction.[103] In this study of 994 AF patients who were enrolled in the SPAF III trial, the $CHADS_2$ and Birmingham risk stratification schemes both predicted ischaemic stroke and vascular events but addition of high vWf levels to both clinical risk scores further refined risk stratification.[103]

Recently biomarker-based risk scores have been developed for improved risk prediction in AF such as the ABC [Age, Biomarkers (troponin and NT-proBNP), and Clinical history of stroke/TIA] stroke risk score. In recent sub-studies of anticoagulated clinical trial cohorts, the ABC score has outperformed the current validated CHA_2DS_2-VASc score for stroke risk prognostication with c-indices of 0.65–0.68 (ABC) vs. 0.60–0.62 (CHA_2DS_2-VASc); $P \leq 0.004$.[199,200] While statistically significant, all c-indexes were <0.7 indicating only modest predictive value. In real world cohorts, especially with long-term follow-up, the ABC score and the addition of other multiple biomarkers did not confer added predictive advantage over the CHA_2DS_2-VASc score.[201,202]

Similarly, a biomarker-based risk score for prediction of major bleeding in AF, the ABC-bleeding score including ABC of prior bleeding has been proposed, and shown to perform better than previously validated HAS-BLED and ORBIT scores.[203] Again, while statistically significant, all c-indexes were <0.7 indicating only modest predictive value. In real world cohorts, especially with long-term follow-up, the ABC-bleeding score did not confer added advantage over the HAS-BLED score.[204] GDF-15 is also non-specific and has been related to other adverse outcomes in cardiovascular disease[205–207] and the progression of glaucoma.[208]

6.1 Where do we stand with biomarkers associated with prothrombotic state in AF?

Biomarkers will always be additive and improve on the predictive value of clinical factor based risk scores, whether in AF or non-AF patients. The issue is whether measuring single or multiple biomarkers will improve clinical management, balancing simplicity and practicality of decision-making in busy outpatient clinics or wards.

There are relatively limited data on the change in risk profile over time, and the relation to biomarker changes. Indeed, stroke and bleeding risks in AF patients are dynamic, and a change in risk factor profile is more predictive of outcomes than baseline risk assessment whether for stroke[209,210] or bleeding.[211] Thus far, most studies looking at biomarkers in AF have been based on single measurement at study entry, with outcomes determined many years later when the patient would be older and have acquired risk factors or had drug therapy changes. Repeated measurements may provide additional useful information such as determinants for increase of these biomarkers and subsequent risk in AF.

Indeed, sustained or incremental increases of troponin and NT-proBNP concentrations over time are associated with cardiovascular comorbidities and confer an even higher risk of stroke and mortality.[212] Results from The British Regional Heart Study, a prospective study of 3366 men followed up for a mean period of 13 years, showed that D-dimer and vWF were significantly and independently associated with NT-proBNP.[213] This implies that increased coagulation activity may be related to cardiac stress and consequent neurohormonal activation.[213] This has been supported by work showing that D-dimer levels can be elevated in AF patients with few clinical risk factors but high-BNP levels.[214] In AF patients, elevated NT-proBNP is associated with unfavourably altered prothrombotic fibrin clots and this phenotype is associated with prior ischaemic stroke.[215] Indeed, NT-proBNP levels can serve as a significant marker of LA thrombus in AF patients with acute ischaemic stroke or TIA.[216] Furthermore, Maruyama *et al.*[217] have shown that in patients with non-valvular AF and acute ischaemic stroke, apart from National Institute of Health Stroke Scale (NIHSS), BNP was a very useful predictor for long-term outcomes.

New biomarkers of the prothrombotic state have also been investigated. As one recent example illustrates, Rivera-Caravaca *et al.*[218] investigated the association and predictive performance of soluble fibrin monomer complex (SFMC) for stroke, adverse cardiovascular events, mortality in a cohort of patients with AF receiving vitamin K antagonist anticoagulant therapy. They showed that SFMC >12 μg/mL was not associated with stroke but was associated with higher risk of cardiovascular events and mortality. When this was combined with CHA_2DS_2-VASc score, there was (as expected) significant improvement in predictive performance with c-index of 0.661 (without) vs. 0.691 (with) ($P < 0.001$) but decision curves demonstrated a similar net benefit and clinical usefulness. They concluded that the addition of SFMC to CHA_2DS_2-VASc score does improve its predictive ability for outcomes (at least statistically) but at the cost of less simplicity, practicality and clinical usefulness.

In summary, the utility of clinical risk scores would always be augmented by biomarkers that can include blood markers (e.g. vWf, D-dimer, troponin, natriuretic peptides), urine (e.g. eGFR, creatinine clearance or proteinuria), cardiac imaging (echocardiography, either transthoracic or transoesophageal), and/or cerebral imaging (e.g. computed tomography or magnetic resonance imaging) which can provide incremental predictive value for the identification of 'high risk' subjects.[219] Such a single- or multi-biomarker approach would probably be at the cost of less simplicity and practicality, limiting its (immediate) 'quick' use in everyday clinical practice. A possible role may well be in AF patients who are 'borderline' decisions for anticoagulation, for example those with CHA_2DS_2VASc 0–1 where biomarker assessment may help decision-making. Biomarkers along with other risk stratification scores are there to aid clinicians in their decision on anticoagulation and follow-up strategies.

7. Conclusion

The evidence shows that AF fulfils Virchow's triad for thrombogenesis with evidence of abnormal changes in flow, vessel wall (atrial structure) and blood constituents. The mechanisms underlying thrombogenesis in AF are complex and only remain partly understood. Assessment of the prothrombotic state in AF gives insight into its pathophysiology, and the pathogenesis of thrombosis in this common arrhythmia.

The prothrombotic state in AF has broader implications for its management. The advent of biomarkers has opened the field for some personalization of treatment for individual patients in AF, given the potential to identify better those at high risk of stroke from those at low risk and give anticoagulation appropriately.

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