Pathophysiology of Haemostasis and Thrombosis

Atrial Fibrillation and the Hypercoagulable State: From Basic Science to Clinical Practice

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Atrial fibrillation (AF) is the commonest sustained cardiac rhythm disturbance in clinical practice [1]. Its presence increases mortality by two-fold [2], mostly by increasing the risk of stroke and thromboembolism. The reasons for stroke occurring in AF are multiple and complex, but many strokes related to AF represent embolism of thrombus formed within the left atrium (LA), predominantly within the left atrial appendage (LAA). The elucidation of the pathophysiological mechanisms leading to stroke and thromboembolism in AF have therefore gained much interest.

Over 150 years ago, Virchow proposed a triad of abnormalities that predisposes to thrombus formation - flow abnormalities, abnormalities of the vessel wall and abnormal blood constituents. The pathophysiology of thromboembolism is AF is multi-factorial but increasing evidence points to the fulfillment of Virchow's triad in this arrhythmia, leading to a prothrombotic or hypercoagulable state in AF.

Flow Abnormalities

The loss of atrial systole in AF results in increased stasis of blood within the left atrium. Fast ventricular rate, which is often a consequence of AF, reduces effective ventricular filling and further worsens intra-atrial stasis. LA stasis may be visualized on the trans-oesophageal echocardiogram (TOE) as spontaneous echocontrast (SEC). This phenomenon is a result of increased interaction between erythrocytes and fibrinogen [3] and has been shown to independently predict increased stroke and thromboembolic risk [4]. Other studies have shown reduced Doppler flow velocities in the LAA in AF, which in turn has been shown to be associated with the presence of LA thrombus, SEC and increased risk of subsequent thromboembolism [5].

A clustering of thromboembolic events has been noted around the time of onset of AF and during transition from paroxysmal AF (PAF) to permanent AF. These observations suggest that a change in rhythm and maybe, the resulting left atrial stasis does play a role in thromboembolism, although the relative contribution to thrombogenesis and embolism remains uncertain.

Abnormalities of Vessel Wall

Despite the above evidence, the risk of stroke in 'lone' AF is relatively low, suggesting blood flow abnormalities per se may not be sufficient for LA thrombus formation. Another aspect of the Virchow's triad - vessel wall abnormalities, are also a feature of AF.

At a macroscopic level, LA and / or LAA enlargement are common findings in AF, can be considered as evidence of vessel wall abnormalities that have been shown to predict subsequent thromboembolism [6]. However, these abnormalities are often secondary to AF itself or associated comorbidity (eg. hypertension).

However, vessel wall abnormalities at the microscopic and molecular levels have also been demonstrated. For example, atrial endocardial damage has been shown on scanning electron microscopy, in the atrial appendages of patients with mitral valve disease, especially in the presence of AF [7]. Masawa et al. [8] described 'rough endocardium', which had a macroscopically wrinkled appearance due to oedematous and fibrous thickening, with small areas of endothelial denudation and thrombotic aggregation visible by scanning electron microscopy in necropsy specimens of left atrial tissue, from patients with AF and cerebral embolism. In another necropsy study, Shirani et al. [9] described a significantly higher prevalence of LAA endocardial fibroelastosis in AF compared to sinus rhythm. Other changes in the form of myocyte hypertrophy, myocyte necrosis, mononuclear cell infiltrate and non-specific fibrotic changes have also been reported [10]. These myocyte changes may in part account for the loss of atrial systole in AF and for the delay in return of atrial systole (termed 'atrial stunning') after successful cardioversion of AF.

It is possible that AF may persist due to structural changes in the atria that are promoted by inflammation. An initial case control study of 131 patients with AF reported elevated CRP levels (an index of inflammation) in AF [11]; of particular interest was the stepwise CRP elevation with higher AF burden [for example, persistent AF had higher CRP than PAF (P=0.008), figure 1]. In a longitudinal study, there was an association between raised baseline CRP levels and the risk of future development of AF (figure 2) [12]. In contrast, Roldan et al. [13] failed to show any association between inflammation (as measured as interleukin-6) with endothelial activation or the presence of abnormal thrombogenesis in AF [13].

Statin therapy has been shown to reduce inflammation in atherosclerosis, diabetes mellitus and hypertension, which
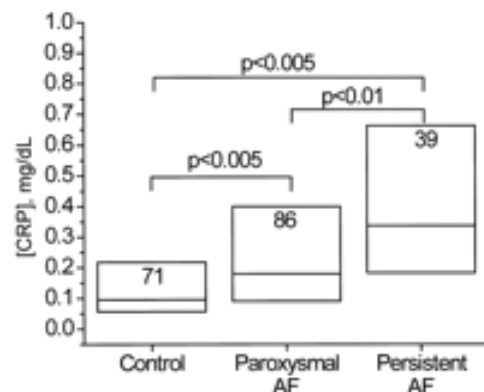
Fig. 1. Step-wise elevation of CRP with increasing burden of AF [11]. Middle line indicates median; bottom of box, 25th percentile; and top of box, 75th percentile. Number of patients in each group is indicated within the box.

Fig. 2. Higher probability of new-onset AF, in patients with higher base-line CRP (median CRP of 1.92 mg per litre). Subjects above median CRP value had lower AF-free survival (P<0.001) [12].
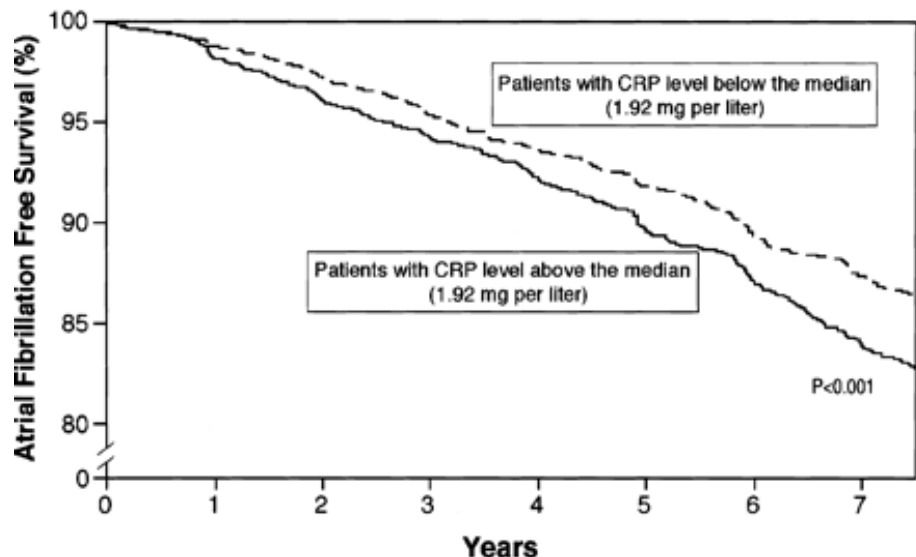
are all increasingly recognized to be pro-inflammatory, pro-thrombotic states. Nonetheless, the use of statins in primary prevention of AF and in its recurrence post-successful cardioversion has shown mixed results [14-17]. Further studies are needed to elucidate whether background inflammation is a consequence of AF itself or its associated comorbidities. It is possible that the increased levels of plasma IL-6, CRP, and plasma viscosity support the case for the existence of an inflammatory state among "typical" populations with chronic AF. Whilst these indices of inflammation are related to some indices of the prothrombotic state, they may be related to the clinical variables of the patients (eg. underlying vascular disease and co-morbidities), rather than simply to the presence of AF itself [18,19].

Furthermore patients with AF have been noted to have impaired matrix degradation, with abnormalities in matrix metalloproteinase-1 (MMP-1) and its inhibitor, tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), although these again were not independently associated with the presence of AF on multivariate analysis [20]. However, an independent relationship was noted between the MMP/TIMP system and the prothrombotic state (assessed by prothrombin fragments 1 and 2 levels). From the available data, it seems that increased interstitial fibrosis in atrial tissue is more likely due to underlying comorbidities, like hypertension, ischemic heart disease, or uncontrolled heart failure (circumstances associated with high risk to develop AF), rather than the presence of the arrhythmia itself.

In 2001, Fukuchi et al. [21] demonstrated increased endocardial expression of vWF in patients with overloaded LA, with correlations between endocardial expression of vWF and adherent platelet thrombus. Later in 2003, Nakamura et al. [22] demonstrated increased expression of tissue factor (TF) in the atrial endocardium, suggesting that local inflammation was involved in the pathogenesis of thrombosis in AF. Indeed, this might indicate a key role of TF in the generation of a prothrombotic state in AF [23], linking the atrium to (systemic) plasma abnormalities, especially since vWF is well-accepted as a marker of endothelial damage/dysfunction.

It is of note that studies investigating the recovery /improvement of endothelial function following successful cardioversion have showed mixed results[24,25], again suggesting endothelial abnormalities in AF might depend more on associated co-morbidities (hypertension, diabetes mellitus, hyperlipidaemia etc) which also influence the endothelium.

Abnormalities of Blood Constituents

The final part of the Virchow's triad, namely abnormal procoagulant blood constituents, is well recognised in AF.

In simple terms, the known intravascular promoters of thrombus formation are platelets and the proteins of the coagulation cascade via their interaction with each other and the vascular endothelium.

Evidence supporting increased platelet activation in AF has been provided by numerous studies [26-31]. Some results are conflicting, thus reflecting the difference between the assays and the different aspects of platelet activation, as measured by them. Though majority of the evidence suggests that platelets are activated in AF, the evidence that it relates directly to the increased thrombotic risk in AF is uncertain. For example, Heppel et al reported an independent association between beta-thrombuglobulin (a marker of platelet activation) and intra-atrial thrombus on TEE [32], a subsequent much larger study [26] found no association between plasma beta-thromboglobulin levels and subsequent thromboembolic events. Furthermore, plasma levels of P-selectin were unrelated to estimated stroke risk among patients in the Stroke Prevention in Atrial Fibrillation III (SPAF III) trial [33], despite associations between soluble P-selectin levels and atherothrombotic risk factors, such as smoking and peripheral vascular disease.

In an interesting study published in 2003, Chung et al. [34] reported increased levels of Vascular Endothelial Growth Factor (VEGF) in AF patients. It is well known that VEGF is largely produced by activated platelets [35], and causes up regulation of TF mRNA and its subsequent expression on the endothelial cell membrane [36]. TF acts as a cofactor to factor VIIa, and the TF-factor VIIa complex activates factors IX and X, thereby triggering the coagulation cascade. Not surprising, Chung et al. [34] not only reported significantly increased levels of TF in the same group of patients, but also noted a significant correlation between TF and the levels of VEGF and VEGF receptor, sFlt-1. Thus, although platelets are activated in AF, it is not directly related to thrombus formation (unlike in coronary artery disease and peripheral vascular disease states) but may play an important role in rendering AF more 'hypercoagulable'.

In contrast, elevated levels of plasma markers of coagulation activation - prothrombin fragments 1+2 (F1+2), thrombin-antithrombin complexes (TAT) and fibrin turnover (D-dimer) - have consistently been reported in patients with chronic AF [37-39]. In addition, impaired fibrinolysis as evidenced by elevated levels of tissue plasminogen activator (t-PA) antigen and tissue plasminogen activator inhibitor type-1 (PAI-1) antigen, have also been noted in such patients [40,41]. Similar abnormalities of coagulation and fibrinolysis have been previously reported to have a predictive value for cardiovascular [42-47] and venous thrombotic events [48, 49]. More recently, Vene et al. [50] have suggested high levels of D-dimer and t-PA antigen are signifi-
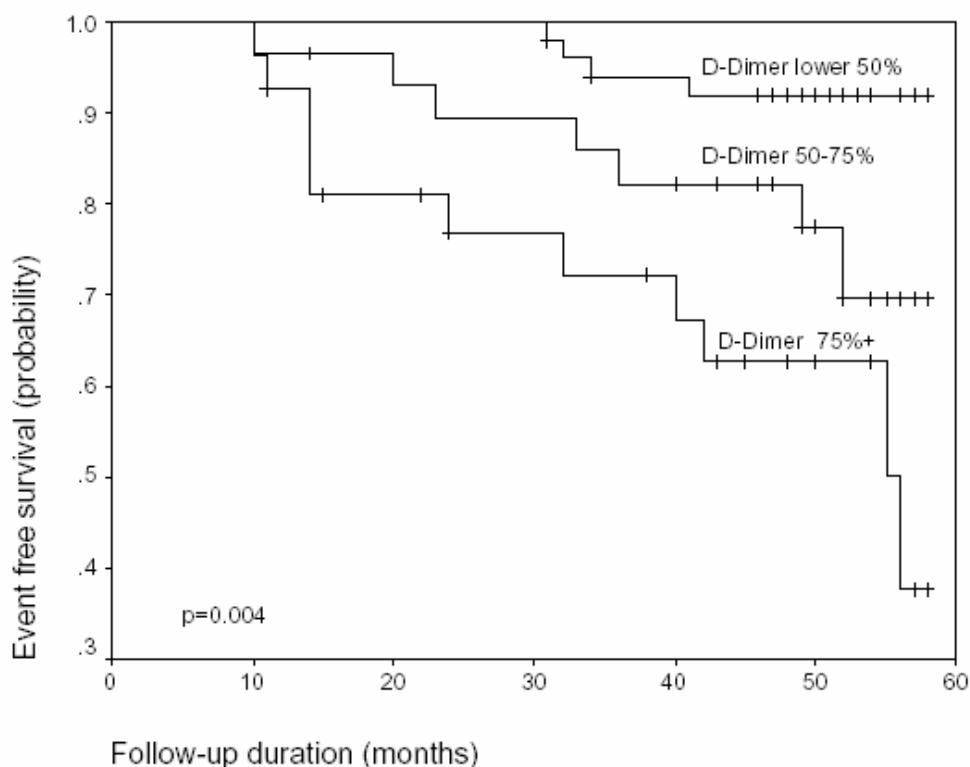
Fig. 3. Cardiovascular event survival curves for D-dimer levels during oral anticoagulation treatment (OAT). The hazard of cardiovascular events increases with increasing plasma D-dimer concentrations with a hazard-ratio about five-fold greater in the highest quartile than in the lowest two quartiles [50].
(75%+) = Top quartile
(50-75%) = third quartile
(lower 50%) = lower two quartiles combined

cant predictors of combined cardiovascular events in AF patients and, on this basis, could perhaps be useful markers of thromboembolic risk stratification in AF (figure 3).

Another particularly important procoagulant factor is vWF due to its ability to interact with platelets and factor VIII [51], as an index of endothelial damage/dysfunction and as a poor prognostic marker in cardiovascular disease. Indeed, Lip et al [39] reported a significant correlation between vWF and plasma levels of D-dimer, whilst Heppel et al [32], in addition to their findings of raised beta-thromboglobulin, found raised levels of vWF to be predictive of the presence of LAA thrombus. Among the 1321 participants in the SPAF III trial , plasma vWF level was associated with the presence of four stroke risk factors independently of each other (heart failure, previous stroke, increasing age and diabetes) [52]. Follow-up data from this study suggested tha vWF levels may even have an independent predictive value for subsequent cardiovascular events [33].

Despite the evidence of abnormal haemostatic factors in AF, AF itself is an extremely heterogeneous condition, often reflecting underlying (at times undiagnosed) cardiovascular pathologies that might themselves account for the abnormalities seen. Although some studies have adjusted for these confounders, it is often difficult to do so, in view of the small number of cases in these studies.
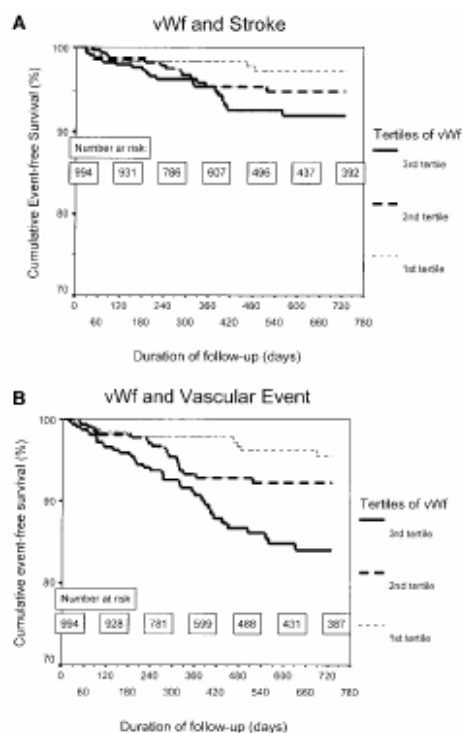
Fig. 4. Plasma vWF levels in NVAF may predict future stroke (A) and vascular events (B) (ischaemic stroke, myocardial infarction, vascular death), with the greatest risk among those with the highest levels of vWF. [50].
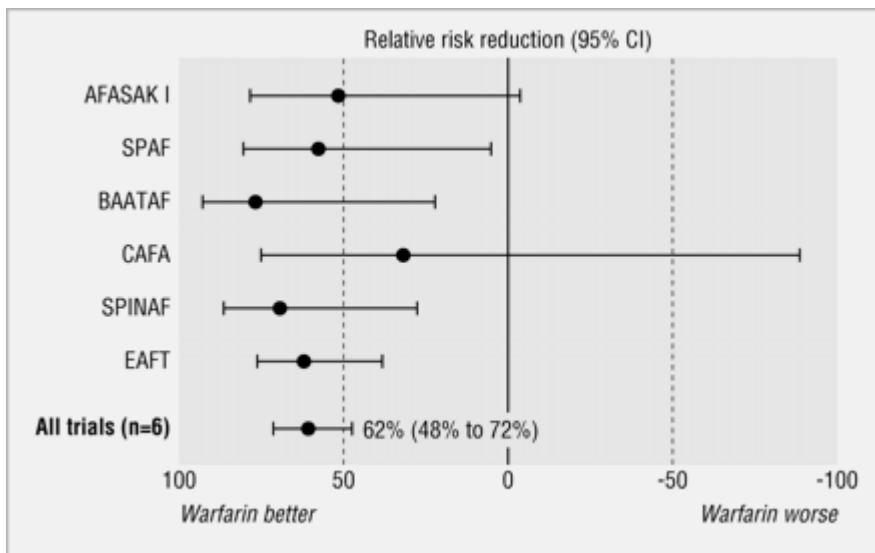
Fig. 5. Meta-analysis of trials comparing warfarin with placebo in reducing the risk of thromboembolism in patients with atrial fibrillation [56].
AFASAK = Copenhagen atrial fibrillation, aspirin, and anticoagulation study; BAATAF = Boston area anticoagulation trial for atrial fibrillation; CAFA = Canadian atrial fibrillation anticoagulation study; EAFT = European atrial fibrillation trial; SPAF = Stroke prevention in atrial fibrillation study; SPINAF = Stroke prevention in non-rheumatic atrial fibrillation
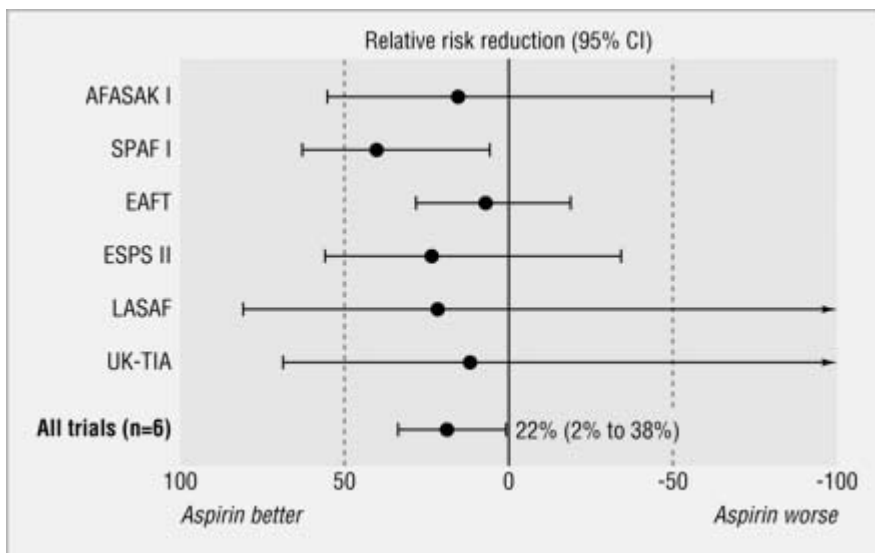
Fig. 6. Meta-analysis of trials comparing aspirin with placebo in reducing risk of thromboembolism in patients with atrial fibrillation [57].
AFASAK = Copenhagen atrial fibrillation, aspirin, and anticoagulation study; EAFT = European atrial fibrillation trial; ESPS II = European stroke prevention study II; LASAF = Low-dose aspirin, stroke, and atrial fibrillation pilot study; SPAF = Stroke prevention in atrial fibrillation study; UK-TIA = United Kingdom TIA study

In a large epidemiological study, the Framingham Offspring Study, raised levels of haemostatic markers among AF cases compared to the general population became non-significant when additional cardiovascular pathologies were accounted for [53]. However, the numbers of cases of AF were small (n=47), and in view of more recent findings, a true association between AF and some of the haemostatic markers is not in doubt. Furthermore, there does not appear to be any significant circadian or diurnal variation in the haemostatic markers of AF [54], suggesting that a persistent hypercoagulable state in AF exists. Nonetheless, the relationships between haemostatic markers, AF, cardiovascular co-morbidities and the risk of stroke need further evaluation to determine which markers are related to AF per se, which

are related mainly to the co-morbidities and which might be useful in risk stratification in AF.

Clinical Correlations of Thromboembolism in Atrial Fibrillation

The presence of NVAF increases the risk of stroke approximately fivefold [53]. This risk increases to about 12% in patients with a past history of transient ischaemic attack (TIA) or stroke [55]. In the presence of mitral stenosis and AF there is an approximately eighteenfold increase in stroke risk, but the declining prevalence of rheumatic heart disease means that non-valvular AF represents the greatest public health problem.

The risk of thromboembolic stroke due to AF also rises with age, and thus AF becomes the predominant independent risk factor for stroke in the age group of 80-89 years, with an attributed risk of 23.5% of all strokes [55]. Indeed, AF is present in approximately 20% of all stroke patients, and stroke associated with AF carries an increase in mortality, morbidity and healthcare costs. Nonetheless, the risk of stroke in AF is not homogeneous, and the presence of certain additional clinical and echocardiographic features are also associated with an increase in AF-related stroke risk, and may be useful to identify patients at greatest risk in order to target appropriate therapy, allowing risk stratification for thromboprophylaxis [Table 1]. Importantly, PAF seems to carry the same risk as those with persistent AF. The same criteria can be used to identify high risk patients, although it is unclear whether the risk is dependent on the frequency and duration of the symptoms.

One meta-analysis of antithrombotic therapy in AF has shown that adjusted dose warfarin to reduce stroke by about 60%, with absolute risk reductions of 3% a year for primary prevention and 8% a year for secondary prevention (numbers needed to treat for one year to prevent one stroke of 33 and 13, respectively) (figure 4) [56]. In contrast, aspirin reduces stroke by about 20%, with absolute risk reductions of 1.5% a year for primary prevention and 2.5% a year for secondary prevention (numbers needed to treat being 66 and 40, respectively) (figure 5) [57]. Relative to aspirin, adjusted dose warfarin reduced the risk by about 40%, and the relative risk reduction was similar for primary and secondary

prevention, and for disabling and non-disabling strokes.

The effects of aspirin seem to be on smaller non-disabling strokes rather than disabling strokes. This may be due to an effect primarily on carotid and cerebral artery platelet thrombus formation, rather than on formation of intra-atrial thrombus. The SPAF III trial demonstrated that addition of fixed low doses of warfarin to aspirin treatment is not sufficient to achieve the benefits of full dose warfarin alone.

As stated earlier, there seems to be a clustering of thromboembolic events around the time of onset of AF and transition from PAF to permanent AF. This is also true when there is transition from AF to sinus rhythm (in PAF or following cardioversion), with approximately 7% risk of thromboembolism associated with DCC if anticoagulation is not properly used. There is no hard evidence to suggest that restoration of sinus rhythm reduces risk of subsequent stroke. Even in cases of successful cardioversion thromboembolic risk persists for a few weeks postprocedure, and continuation of warfarin for at least four weeks is recommended, and in patients with risk factors or high chance of AF recurrence, consideration should be given towards long term anticoagulation post-cardioversion. Thus, long term continuation of warfarin should be guided by the overall risk rather than success of cardioversion. Cardioversion should not be attempted with an aim to stop anticoagulation per se as this often leads to an increased incidence of stroke, especially with recurrence (frequently asymptomatic) of AF in patients with associated risk factors for stroke.

Despite the obvious superiority of wafarin over aspirin,

Table 1. Risk stratification and anticoagulation in nonvalvular atrial fibrillation (Adapted from Lip et al BMJ 2002;325 :1022-5)

Assess risk and reassess regularly

1. High risk (annual risk of CVA=8-12%)
    o All patients with previous transient ischaemic attack or cerebrovascular accident
    o All patients aged 75 and over with diabetes and/or hypertension
    o All patients with clinical evidence of valve disease, heart failure, thyroid disease, and/or impaired left ventricular function on echocardiography*

2. Moderate risk (annual risk of CVA=4%)
    o All patients aged under 65 with clinical risk factors: diabetes, hypertension, peripheral arterial disease, ischaemic heart disease
    oAll patients aged over 65 who are not in high-risk group

3. Low risk (annual risk of CVA=1%)
    o All other patients under 65 with no history of embolism, hypertension, diabetes, or other clinical risk factors.

Treatment
High risk: give warfarin (target INR 2.0-3.0) if no contraindications and possible in practice.
Moderate risk: either warfarin or aspirin 75-300 mg. In view of insufficient clear-cut evidence, treatment may be decided on individual cases. Referral and echocardiography may help.
Low risk: give aspirin 75-300 mg daily

*Echocardiography-not needed for routine risk assessment but refines clinical risk stratification in case of impaired left ventricular function and valve disease. A large atrium per se is not an independent risk factor on multivariate analysis
[CVA = cerebrovascular accident]

Table 2. Efficacy and safety outcomes of the SPORTIF III and V trails: ximelagatran versus warfarin in nonvalvular atrial fibrillation [60, 61].

Outcomes	SPORTIF III			SPORTIF V		
	Ximelagatran [N=1704] n (%)	Warfarin [N=1703] n (%)	RRR (95% CI)	Ximelagatran [N=1960] n (%)	RRR (95% CI)	Warfarin [N=1962] n (%)
Stroke or systemic embolism	40 (2.3)	56 (3.3)	0.71 (0.48-1.06)	51 (2.6)	1.38 (0.91-2.10)	37 (1.9)
Major bleeding	29 (1.7)	41 (2.4)	0.71 (0.44-1.13)	63 (3.2)	0.75 (0.54-1.03)	84 (4.3)
ALT increase of >3 times upper limit	107 (6.3)	14 (0.8)	7.64 (4.39-13.28)	118 (6.0)	7.38 (4.40-12.40)	16 (0.8)

N: Number of patients; n: Number of events; ALT: Alanine Transaminase; RRR: Relative Risk Reduction; ARR: Absolute Risk Reduction; CI: Confidence Interval

only one-fourth to one-third of the eligible patients land up on warfarin [58-60]. This is mostly due to the inconvenience of regular INR monitoring, and multiple drug and food interactions with warfarin, resulting in an increased risk of bleeding. With many new antithrombotic agents in development, ranging from the direct oral thrombin inhibitors to oral Factor Xa inhibitors, it is clear that research into indices of the prothrombotic state in AF may finally be coming of age, whereby crucial coagulation pathways in AF that con-tribute to the prothrombotic state in this condition (and others) may be identified and targeted.

In order to reap the benefits of our increasing and better understanding of the pathophysiology of AF, increased emphasis should perhaps also be placed on identifying patients with AF in the community [61] followed by appropriate treatment.

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