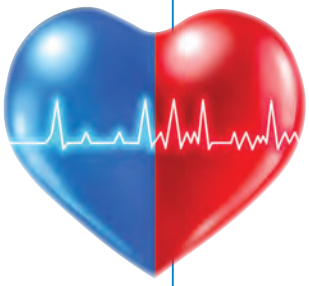
1 Coagulative patterns in atrial fibrillation



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INTRODUCTION

Atrial fibrillation (AF) is defined as a supraventricular tachyarrhythmia with uncoordinated atrial electrical activation and consequently ineffective atrial contraction.[1] It is the most common sustained cardiac arrhythmia in adults, with a prevalence comprised between 2% and 4%, and an estimated lifetime risk of occurrence of 1 among 3 individuals. AF is associated with substantial morbidity and mortality burden, especially due to stroke and systemic embolic events derived from embolism of thrombotic formations developed in the left atrium (LA) or mainly in the left atrial appendage (LAA).[2] Overall, AF carries a 5-fold increase in the risk of stroke compared to people with normal sinus rhythm, and off all patients with an ischemic stroke, 20-30% would have AF.[3] In AF the occurrence of embolic events has been demonstrated to be strongly dependent on a higher risk of thrombus formation in the LAA or LA caused by disruption of normal hemostatic mechanisms. Virchow's triad (venous stasis, endothelial injury, and hypercoagulability) summarizes the classical combination of factors that were proposed more than 150 years ago to describe the pathogenesis of venous thrombosis. Extensive abnormal changes of these variables are also clearly evident in patients with AF, so that a revisitation on the Virchow's triad has been proposed to explain the mechanisms of thrombogenesis in this setting.[4,5] Of note, in AF patients abnormal blood stasis, endothelial damage and hypercoagulability should not be interpreted as independent entities, as they are rather strongly interconnected to each other and influenced by comorbidities environment, causing the so called "atrial cardiomyopathy" (AC), as depicted in **Figure 1.1** and discussed below.

ATRIAL CARDIOMYOPATHY AND THE ROLE OF COMORBIDITIES

AC is defined as: "*any complex of structural, architectural, contractile, or electrophysiological changes affecting the atria with the potential to produce clinically relevant manifestations.*"[6] AC is a key substrate for AF, and is characterized by LA remodeling with dilation and fibrosis, but also by endothelial dysfunction, thus creating an ideal prothrombotic milieu. Despite various genetic

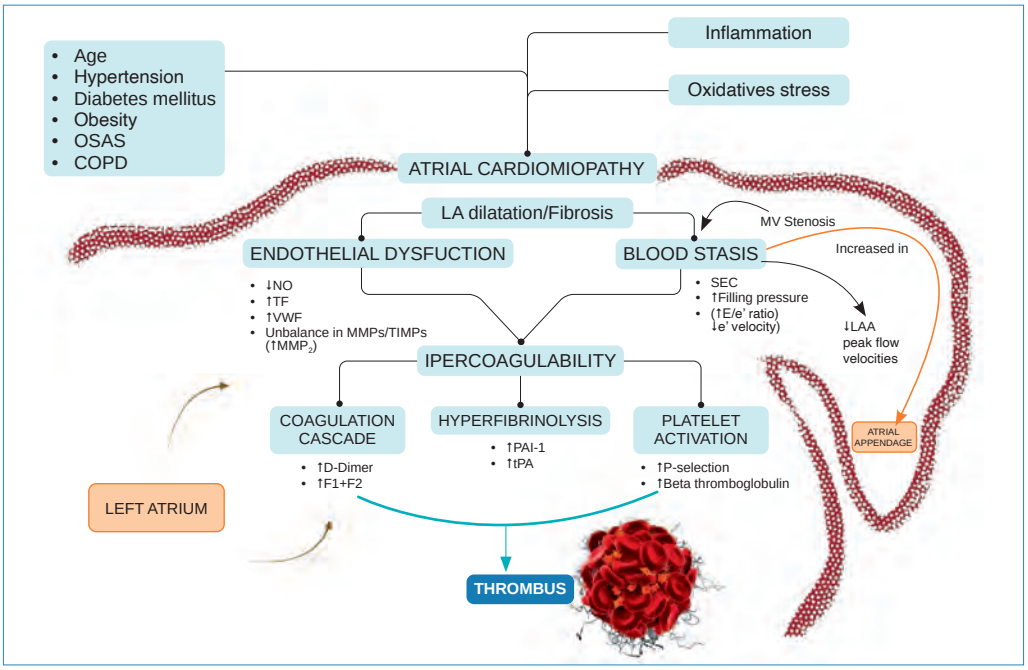

Figure 1.1. Revisitation of the Virchow's triad in atrial fibrillation. COPD: chronic obstructive pulmonary disease; F1+F2: Prothrombin fragment 1 + 2; LAA: left atrial appendage; MMP: Matrix metalloproteinase; MV: mitral valve; NO: nitric oxide; OSAS: obstructive sleep apnea syndrome; PAI-1: plasminogen activator inhibitor-1; SEC: spontaneous echocardiographic contrast; TF: issue factor; TIMP: Tissue inhibitor of metalloproteinase; t-PA: tissue-plasminogen activator; vWF: von Willebrand factor.

determinants have been also investigated, acquired cardiovascular risk factors have a pivotal role in the pathogenesis of AC.[7] LA dilation in patients with older age, obesity, diabetes mellitus, arterial hypertension and/or sleep apnea is usually considered as a consequence of diastolic dysfunction, but a direct independent effect of such clinical conditions may have also a role.[8] Besides, obesity and diabetes are associated with expansion of the epicardial adipose tissue, which has a strong correlation with atrial myopathy.[9] In this setting, a particular role is played by inflammation, which has been demonstrated to be involved in the initiation and perpetuation of AF, but also in AF-related endothelial dysfunction and thrombogenesis.[10, 11] The proinflammatory environment commonly observed in patients with hypertension and diabetes mellitus results in reactive fibrosis through different pathways, such as renin-angiotensin aldosterone system, beta-adrenergic system, excessive reactive oxygen species and metabolic disturbances caused by hyperglycemia.[12, 13] Indeed, the higher levels of C-reactive protein and interleukin-6 reported in patients with AF can promote tissue factor production and thus thrombus formation.[14]

BLOOD STASIS

The abnormal blood flow within the LA observed in patients with AF is caused by several mechanisms, overall resulting in atrial hypo-contractility. First of all, AF itself causes loss of the atrial systole, which contributes to ventricular filling. Moreover, LA structural changes (enlargement and fibrosis) have been consistently demonstrated to be predictors of both AF and AF-related

stroke occurrence.[15-17] Indeed, the assessment of LA function by strain and strain rate imaging demonstrated that a reduced LA reservoir function correlates with LA fibrosis at cardiac magnetic resonance and is an independent risk factor for stroke.[18, 19] Flow LA abnormalities in AF may also be exacerbated in presence of mitral stenosis, that increases LA size, reduces LA emptying, and further promotes thrombogenesis. Finally, it is well-known that, among patients with AF, approximately 90% of thrombi originate in the LAA, where blood stasis is enhanced.[20, 21] Histological examinations of the LAA found endocardial fibroelastosis causing a reduction in pectinate muscle volume.[22] Furthermore, a "LAA rough endocardium" was documented in AF patients who died from cerebral embolism.[23] Overall, a confirmation of the role of abnormal blood flow in LAA thrombogenesis derives from echocardiographic studies. In particular, imaging surrogates for stasis, such as spontaneous echocardiographic contrast, increased filling pressures (high E/e' ratio, low e' velocities) and reduced LAA peak flow velocities (*e.g.*, all features being more prevalent in presence of a cauliflower or cactus LAA shapes, where the presence of trabeculae is greater), were independent predictors of stroke in AF.[24-26]

ENDOTHELIAL DYSFUNCTION

Endothelial dysfunction is the cornerstone of thrombogenesis in patients with AF, where scanning electron microscopy revealed larger areas of endothelial denudation and thrombotic aggregations within the LA.[22] Endothelial dysfunction may be due to both AF *per se* and other underlying comorbidities. Notably, several biomarkers have been involved in this regard. First of all, animal models of AF showed that the loss of atrial contraction and consequent reduction in shear stress is associated with lower endocardial nitric oxide (NO) synthase expression, with corresponding decrease in NO bioavailability.[27] Besides, higher levels of asymmetric dimethylarginine (an endogenous inhibitor of NO) were found in patients with AF compared to those in sinus rhythm. This could result in thrombus formation, as NO has potent antithrombotic effects by inhibition of platelet activation and increased expression of the tissue-plasminogen activator inhibitor (PAI-1).[28, 29] Furthermore, endothelial sites containing inflammatory cells and denuded endocardium expose higher levels of tissue factor and von Willebrand factor (vWf), as demonstrated in AF patients, especially in presence of more severe LA remodeling.[30, 31] There is also evidence that extracellular matrix homeostasis affects thrombogenesis. Indeed, LA fibrosis is the results of an unbalance between extracellular matrix components production and breakdown, which is mediated by matrix metalloproteinases (MMPs) and their inhibitors (TIMPs). Increased levels of MMP-2 have been found in patients with AF and experimental data showed that such molecule has a role in platelet activation.[32, 33]

HYPERCOAGULABILITY

In the classic view of venous thrombogenesis, as initially described in the Virchow's triad, hypercoagulability results from hereditary thrombophilia or from acquired conditions, such as cancer, systemic inflammation or obesity. In the setting of AF, the alterations of laboratory biomarkers of hypercoagulability should be considered as the result of a process of thrombogenesis, beginning with endothelial damage and blood stasis, as described above. Indeed, higher levels of prothrombin fragment F1+F2, fibrinogen and D-dimer have been demonstrated in AF patients.[34, 35] Importantly, there is a correlation between these parameters and increased spontaneous echocardiographic contrast in the LA or impaired LAA function, confirming the role of blood stasis in

LA/LAA thrombogenesis.[36] An enhanced activation of the coagulation cascade is considered the main expression of hypercoagulability in AF, but increased platelet reactivity and impaired fibrinolysis have also been implicated.[4] Hypo-fibrinolysis, expressed as increased concentrations of PAI-1, was found in patients with AF and it was correlated with the degree of LA enlargement.[37] It is worth notice that in patients with AF more elevated levels of coagulation and fibrinolysis biomarkers were documented in the LAA compared to peripheral samples, thus confirming the presence of a local prothrombotic milieu.[38] On the other side, the role of platelets is debated. Studies reported in patients with AF higher values of P-selectin and β-thromboglobulin, which indicate platelet activation.[39, 40] Notably, these levels were correlated with reduced NO synthesis (*i.e.*, with endothelial dysfunction) and with spontaneous echocardiographic contrast in the LA (*i.e.*, with blood stasis).[41, 42] Furthermore, it has been demonstrated that platelet activation increases <12 hours after AF onset and decreases 24 hours after successful cardioversion.[43] However, other investigations failed to demonstrate any association between platelet activation and thromboembolic events in the setting of AF, which is in accordance with the negative results of randomized trials in terms of prevention of thromboembolic complications with the use of antiplatelet therapy compared to oral anticoagulation.[44, 45] Indeed, some authors reported that enhanced platelet activation in AF is no longer significant after adjustment for underlying comorbities.[46, 47] In contrast, investigations performed on patients with "lone" atrial fibrillation (*e.g.*, without any significant concomitant cardiovascular risk factor) found increased levels of various biomarkers related to both coagulation activation and platelet aggregation, such as P selectin, β-thromboglobulin, fibrinogen, vWf, D-dimer, tissue-type plasminogen activator (tPA) and PAI, as compared with age-matched and sex-matched healthy controls.[48, 49]

CONCLUSIONS

Several factors contribute to the hypercoagulability condition observed in patients with AF. Such prothrombotic state, which is the cause of the thromboembolic risk associated with the disease, results from: concomitant morbidities; endothelial dysfunction and local inflammation, which represent the "*primum movens*" for coagulative activation and are an expression of risk factors for developing AF, as well as of AF *per se*. The effects of this prothrombotic state are amplified in presence of LA/LAA blood stasis. The possibility of measuring these patterns could ameliorate our ability in selecting patients at higher thrombotic risk and in implementing personalized therapeutic approaches to prevent AF-related clinical complications.

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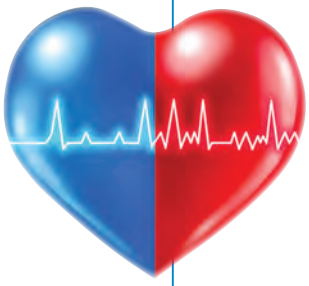
2 Thromboembolic risk stratification in patients with atrial fibrillation



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INTRODUCTION

Atrial fibrillation (AF) patients have a higher risk of thromboembolic events – of which stroke is the most clinically relevant (often disabling or life-threatening) – and oral anticoagulant therapy (OAC) decreases such risk over the short- and long-term.[1-3] Given the intrinsic predisposition to thromboembolism, the risk stratification is a critical component for the clinical management of AF patients. As indicated in Chapter 1, the original, historical model where blood stasis was considered as the primary factor in the left atrium (LA)/left atrial appendage (LAA) thrombogenic process, has been revised in light of the observations that rhythm control strategies do not reduce the risk of AF-related stroke and that the occurrence of thromboembolic events is often unrelated to the onset of AF episodes.[2] Thus, the presence of atrial cardiomyopathy and all the elements of the Virchow's triad (blood stasis, endocardial dysfunction, hypercoagulability) significantly contribute to AF-related thrombosis in LA/LAA.[4-6] In particular, with regard to blood stasis, flow velocities in the LAA <37 cm/s resulted as a powerful predictor of stroke in non-anticoagulated patients with AF.[4] It is generally accepted that thrombus formation in the LAA occurs approximately 48 hours after the onset of AF. This is based on the evidence that performing cardioversion without prior transesophageal echocardiography or prolonged anticoagulation is associated with a very low thromboembolism rate (<1%) when AF onset is <48 hours. A similar incidence has been described in anticoagulated patients undergoing cardioversion.[4] However, the time to *in-situ* thrombus formation may be shorter when the components of Virchow's triad are more pronounced (*e.g.*, severe stasis with spontaneous echo contrast, marked LAA enlargement, severe hypercoagulability, concomitant cardiovascular risk factors), thereby identifying patients at even higher risk of thromboembolism.[4]

SCORES FOR THROMBOEMBOLIC RISK STRATIFICATION

The risk of cerebral or systemic thromboembolic events should be assessed by considering several clinical factors and laboratory data, often combined into risk scores (**Figure 2.1**).

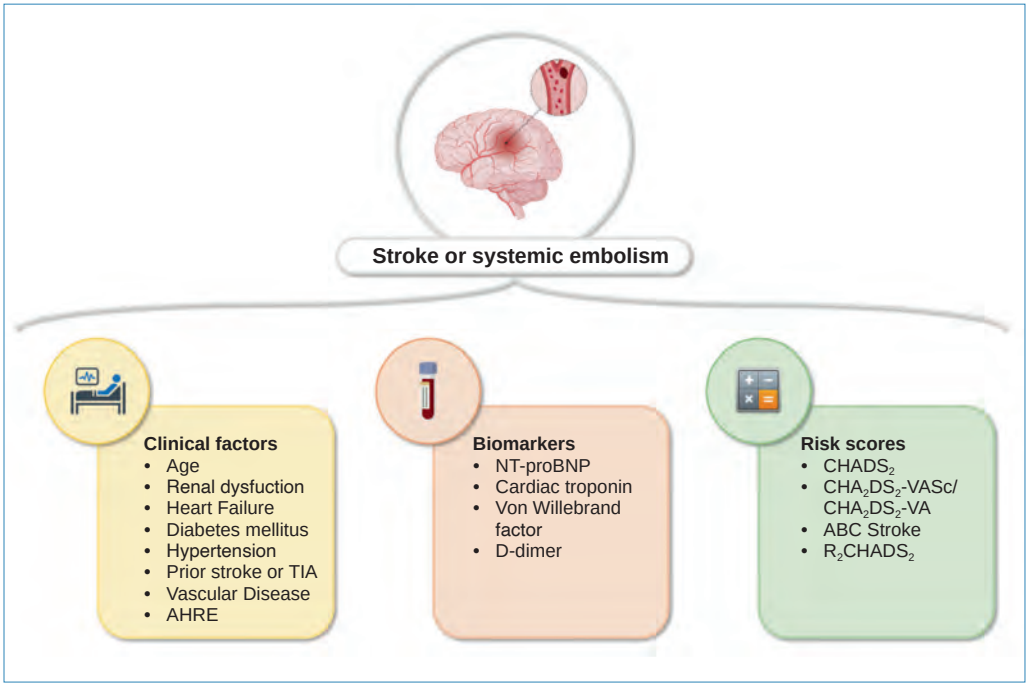

Figure 2.1. Thromboembolic risk stratification in patients with non-valvular atrial fibrillation. AF: atrial fibrillation; TIA: transient ischemic attack; NT-proBNP: N-terminal pro-brain natriuretic peptide; AHRE: Atrial High-Rate Episodes.

CHA_2DS_2-VASc (CHA_2DS_2-VA) and CHA_2DS risk scores

CHA_2DS_2-VASc is the most widely used risk score for predicting thrombotic complications in AF and is currently recommended by European and American guidelines, although its performance is relatively modest.[1, 2] The score consists of nine items, each of which is assigned 1 point, except for previous stroke/transient ischemic attack (TIA)/thromboembolism and age ≥75 years, both of which are assigned 2 points (**Table 2.I**). Of note, systemic hypertension in women and vascular disease in men carry less weight.[7] An earlier version of this score, the $CHADS_2$ score, which included heart failure, hypertension, age ≥75 years, diabetes mellitus, and previous stroke/TIA, has also a moderate predictive ability, but it is less performing than the more recent iteration, especially in patients at low thromboembolic risk.[1] European guidelines recommend the use of OAC in AF patients with CHA_2DS_2-VASc score ≥2 in men or ≥3 in women (class of recommendation I, level of evidence A)[1]. OAC should be considered (IIa B) for stroke prevention in AF patients with a CHA_2DS_2-VASc score of 1 in men or 2 in women, in whom treatment should be individualized based on net clinical benefit and patient values and preferences must be considered.[1] Indeed, the latest European Society of Cardiology (ESC) guidelines introduced the newer CHA_2DS_2-VA score, where the female gender is removed and OAC is recommended in patients with CHA_2DS_2-VA score ≥2 (I A) and is considered in those with CHA2DS2-VA score 1 (IIa B)[8].