Endothelial function and atrial fibrillation: A missing piece of the puzzle?

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1 | INTRODUCTION

The endothelium consists of a single layer of squamous cells which lines both the peripheral vasculature and the endocardial surface of the heart. Far from being an inert lining, the endothelium is a complex endocrine organ with critical roles in regulating vascular tone, hemostasis, and inflammation. It releases vasoactive mediators in response to hemodynamic shear stress, constitutively inhibits the coagulation cascade to prevent intravascular thrombosis, and actively recruits immune cells to sites of tissue injury by altering the expression of adhesion molecules. Endothelial dysfunction, a term used to describe both the physical damage and dysregulated physiology of this endothelial lining, has been implicated in many disease states including atherosclerosis, diabetes, hypertension, and obesity. Over the last 20 years, there has been significant progress in understanding the role of endothelial dysfunction in atrial fibrillation (AF). There is now an emerging role for endothelial dysfunction in promoting and maintaining atrial arrhythmic substrate, inducing atrial thromboembolism, and predicting AF recurrence following cardioversion and ablation therapy. This review will summarize the literature to date and highlight areas for future research.

2 | IS THERE ENDOTHELIAL DYSFUNCTION IN AF?

Three key modalities have been used to assess endothelial function in AF: circulating markers of endothelial dysfunction (Figure 1), microscopic visualization of endothelial structure (Figure 2), and physiological measurements of blood flow (Figure 3).
FIGURE 1  Circulating markers of endothelial dysfunction. 1) Multimeric Von Willebrand factor (VWF) is synthesized within endothelial cells and secreted by the fusion of Weibel–Palade bodies to the cell membrane. Multimeric VWF is cleaved by the enzyme ADAMTS13 into its active form. Exposure of VWF to subendothelial collagen stimulates platelet activation and aggregation. 2) Inactive endothelial nitric oxide synthase (eNOS) is bound to caveolin on the cell membrane. Increased intracellular calcium levels and shear stress increase Ca\(^2+\)/calmodulin-dependent (CaM) kinase and protein kinase A (PKA) phosphorylation of eNOS into its active form. Active eNOS catalyzes the conversion of L-arginine into L-citrulline and nitric oxide (NO). Asymmetric dimethylarginine (ADMA), a methylated analog of L-arginine, acts as a competitive inhibitor of eNOS. 3) Adhesion molecules on the cell surface, including E-selectin, P-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), mediate leukocyte adhesion and recruitment. 4) Circulating microparticles, formed from outpouchings of the endothelial cell membrane, act as vasoactive and procoagulant mediators. They contain phospholipids, enzymes, messenger RNA, and membrane-bound adhesion molecules.

FIGURE 2  Atrial endocardial changes in atrial fibrillation (AF). (A) Masson trichrome stain of human left atrial tissue. Histological changes include endocardial thickening (red arrow) and subendothelial fibrosis and immune cell infiltration (yellow arrow). From Sonada et al.\(^4\) with permission. (B) Scanning electron microscopy of left atrial endocardial surface in non-AF patients. Described as "flat and continuous paving-stone-like arrangement of endothelial cells." From Masawa et al.\(^5\) with permission. (C) Scanning electron microscopy of left atrial endocardial surface in AF patient. The irregular arrangement of endothelial cells with areas of denudation and microthrombi formation. From Masawa et al.\(^5\) with permission.
Circulating markers of endothelial dysfunction include Von Willebrand factor (VWF), nitric oxide (NO), asymmetric dimethylarginine (ADMA), adhesion molecules, and circulating microparticles. VWF is a glycoprotein synthesized and released both by endothelial cells and megakaryocytes (Figure 1). It plays an important role in platelet adhesion and hemostasis following endothelial injury and, therefore, raised plasma levels are a widely used measure of endothelial dysfunction. Studies have shown that VWF levels are higher in patients with AF compared to sinus rhythm. Although raised VWF levels are raised in many disease states which frequently co-exist with AF (hypertension, hyperlipidemia, obesity), AF is associated with raised VWF levels after correction for these confounders and when these confounders are absent. VWF levels have been shown to have a positive correlation with AF burden. Scridon et al. measured plasma levels of VWF from the left atrium, coronary sinus, and periphery in patients with paroxysmal and permanent AF and compared these to sinus rhythm control with Wolff–Parkinson–White syndrome. They showed that VWF levels were significantly increased in patients with persistent AF compared to controls. Patients with paroxysmal AF had raised VWF levels in the left atrium (but not coronary sinus or periphery), while patients with persistent AF had raised VWF levels in all three sites. The authors hypothesize that endothelial dysfunction may be limited to the left atrium in paroxysmal AF and become more widespread as the AF burden increases. This is supported by the observation that VWF levels are positively correlated with the degree of left atrial endocardial damage in patients with AF and mitral valve disease. In patients with AF, raised VWF levels have also been shown to be a predictor of major adverse cardiovascular events (MACEs) and all-cause mortality but not stroke.

Additional circulating markers of endothelial dysfunction include molecules involved in NO metabolism. NO is a vasotransmitter synthesized by nitric oxide synthase (NOS), an enzyme that exists in three isomeric forms (endothelial NOS [eNOS], neuronal NOS, and inducible NOS). Endothelial cells constitutively express eNOS whilst cardiomyocytes express all three forms (Figure 1). NO plays a critical role in regulating vascular tone and the antithrombotic properties of the endothelium, hence reduced NOS expression and low NO levels are widely used measures of endothelial dysfunction. Cai et al. demonstrated that left atrial NO production and left atrial eNOS expression were significantly reduced in a pig model of AF. Plasma levels of nitrite/nitrate (plasma NOx), a surrogate for NO...
levels, are reduced in patients with AF compared to sinus rhythm.23,24 Although most studies support the view that eNOS expression and NO levels are reduced in AF, the 786T/C polymorphism in the promoter of the eNOS gene, associated with reduced eNOS expression, is protective for developing new-onset AF in Caucasians.25 ADMA, a methylated analog of L-arginine, is the precursor of NO.26 It competitively inhibits NOS, and, therefore, raised ADMA levels are a commonly used marker of endothelial dysfunction. Several studies have shown that AF is associated with raised ADMA levels27–30 and that raised ADMA levels predict AF recurrence following cardioversion31 and catheter ablation.32 In addition, raised ADMA levels positively correlate with the CHA2DS2-VASc score29,33 and are strongly associated with increased mortality and stroke in patients with AF.33 One notable exception is data from the Framingham heart study which showed that the association between ADMA levels and new-onset AF became nonsignificant after correction for confounding cardiovascular risk factors.34

Adhesion molecules are expressed on the endothelial surface and mediate leukocyte recruitment as part of the inflammatory response35 (Figure 1). Increased circulating levels of adhesion molecules are used as markers of endothelial dysfunction but studies examining their relationship to AF have been divergent. Some studies have shown raised levels of E-selectin36,37 and vascular cell adhesion molecule-138 in patients with AF. In contrast, Schnabel et al.39 reported no relationship between levels of adhesion molecules and new-onset AF. Circulating microparticles are small membrane-bound vesicles containing glycoproteins, phospholipids, and other cytoplasmic molecules (Figure 1). They are released by endothelial cells and platelets in response to apoptosis and cellular activation and have been shown to play an important role as vasoactive mediators and procoagulants.40 Several studies have shown raised levels of circulating microparticles in AF41,42 and this has been suggested to be a further marker of endothelial dysfunction.

While circulating markers support the view that there is significant endothelial dysfunction in AF, they do not clearly identify whether this dysfunction is localized to the atrial endocardium or alternatively represents more widespread endothelial involvement. Histological studies on human left atrial tissue have shown wrinkled endothelial lining and a thickened subendothelial layer with interstitial fibrosis and immune cell infiltration4 (Figure 2A). Electron microscopy of left atrial appendage tissue from patients with AF showed areas of endothelial desquamation and subendothelial edema43 (Figures 2B,C). Interestingly, Kume et al.44 noted that in a rat hypertension model, atrial endothelial changes occurred only 3 days after abdominal aortic constriction and this was associated with a significant increase in AF susceptibility.

Physiological measurements of blood flow suggest that endothelial dysfunction in AF not only occurs in the atrial endocardium but also in the peripheral circulation. Brachial flow-mediated dilatation (FMD) is an extensively used measure of endothelial function45 (Figure 3A). In brief, an ultrasound probe is used to measure brachial artery diameter while simultaneously inflating a forearm cuff to induce downstream tissue ischemia and vasodilation. After 5 min, the cuff is deflated and the subsequent proportionate increase in brachial artery diameter is measured as a marker of endothelial-dependent vasodilation (Figure 1). The sudden increase in blood flow after releasing the cuff causes an increase in endothelial shear stress and endothelial NO production in the brachial artery which subsequently dilates. Low brachial FMD is, therefore, used as a measure of endothelial dysfunction.45 Studies have shown that patients with AF have lower brachial FMD compared to patients in sinus rhythm.37,46 Moreover, this correlates with AF burden; persistent AF is associated with lower brachial FMD than paroxysmal AF.47 In both the Framingham heart study8 and multiethnic study,50 lower baseline brachial FMD was predictive of new-onset AF (Figure 3C). Moreover, lower brachial FMD is associated with higher cardiovascular events in patients with AF.51 One notable exception to these studies was the Gutenberg health study, in which the relationship between low brachial FMD and AF became nonsignificant after correction for confounding cardiovascular risk factors.52

Beyond the brachial artery, impaired vascular reactivity has also been noted in the coronary circulation of patients with AF.53 Skalidis et al.54 directly compared coronary flow reserve, the ratio of hyperemic to resting coronary blood flow, in the left atrial circumflex artery branch of patients with lone AF and healthy controls. While resting coronary blood flow was comparable between the two groups, coronary flow reserve was significantly reduced in the patients with AF during adenosine-induced hyperemia.54 A similar reduction in hyperemic coronary flow reserve was also noted in patients with AF using 15O-H2O positron emission tomography (PET) imaging.55 (Figure 3D). Reduced coronary flow reserve assessed by 82rubidium PET imaging was independently associated with new-onset AF after adjustment for other cardiovascular risk factors.56 It is important to note that endothelial dysfunction cannot be directly inferred from these studies because they all use adenosine, an endothelial-independent vasodilator, to induce hyperemia. However, Corban et al.57 used low-dose acetylcholine, an endothelial-dependent vasodilator, to measure coronary flow reserve in patients presenting with chest pain and nonobstructive coronary disease. They demonstrated that impaired baseline coronary endothelial function (defined as <50% increase in coronary blood flow in response to acetylcholine) was an independent risk factor for incident AF.57

3 | ENDOTHELIAL DYSFUNCTION—CAUSE OR CONSEQUENCE OF AF?

While there is a strong consensus supporting the presence of endothelial dysfunction in AF, significant controversy remains as to whether it plays a contributory role in AF pathogenesis or alternatively occurs as a secondary consequence of AF (see Figure 2).

In support of the former, baseline endothelial dysfunction as assessed by brachial FMD45,50 and coronary flow reserve57 has been shown to precede and predict incident AF. Moreover, AF has well-established risk factors58 which are independently associated with
endothelial dysfunction, namely aging, obesity, hypertension, diabetes, and ischemic heart disease. Endothelial dysfunction may, therefore, act as the final common pathway linking these risk factors to AF pathogenesis. However, the mechanisms linking endothelial dysfunction to atrial arrhythmic substrate remain incompletely understood. Left atrial endocardial cells to undergo an endothelial–mesenchymal transition in AF and this may contribute towards atrial fibrosis and extracellular remodeling. Endothelial cells also regulate immune cell infiltration and inflammation within the heart muscle. In a rat stroke model, Balint et al. demonstrated that left atrial endothelial dysfunction was associated with increased immune cell infiltration and fibrosis, particularly along the border between the left atrium and pulmonary vein. Finally, endothelial dysfunction is strongly associated with oxidative stress and the overproduction of reactive oxygen species (ROS). ROS are known to be arrhythmogenic by directly affecting cardiac ion currents (e.g., L-type Ca2+ current and late Na+ current) and Ca2+-handling apparatus (e.g., Ca2+/CaM-dependent kinase II). ROS trigger focal activity through early and delayed after depolarizations, promote re-entry through increasing action potential duration heterogeneity, and promote myocardial fibrosis and loss of cardiomyocyte electrical coupling.

In contrast, others have proposed that endothelial dysfunction is largely a secondary consequence of AF. Proponents argue that the irregular heart rate, loss of synchronized atrial contraction, and variable stroke volume reduce turbulence and endothelial shear stress within the atria and blood vessels, thereby reducing the release of endothelial NO and other vasoactive mediators. This view is supported by studies that show endothelial function is restored following a return to sinus rhythm. These views are not mutually exclusive and it is conceivable that endothelial dysfunction both promotes and maintains AF. On the basis of this model, a positive feedback loop exists in which endothelial dysfunction promotes atrial arrhythmic substrate, thereby increasing the risk of AF which, in turn, drives further endothelial dysfunction.

4 | ENDOTHELIAL DYSFUNCTION AND THROMBOEMBOLISM

AF causes thromboembolism and stroke by impacting Virchow’s triad of blood stasis, hypercoagulability, and vessel wall abnormalities. Endothelial dysfunction has been shown to impact all three aspects to promote clot formation (see Figure 2).

First, as previously described, endothelial dysfunction promotes atrial arrhythmic substrate and increases the risk of incident AF. This in turn causes a loss of atrial contraction resulting in static blood flow and clot formation within the left atrial appendage. Fujii et al. demonstrated that endothelial dysfunction, as assessed by reduced reactive hyperemia peripheral arterial tonometry (RH-PAT) index (Figure 3B), was independently associated with static atrial blood flow, as determined by spontaneous echo contrast on transoesophageal echocardiography.

In addition, AF is strongly associated with a procoagulant state; increased levels of clotting factors (factor VIII, fibrinogen), markers of platelet activation (platelet factor 4, β-thromboglobulin, P-selectin), and markers of fibrinolysis (α2-plasminogen activator, plasminogen activator inhibitor-1) have all been described. Endothelial dysfunction induces the release of procoagulant factors (VWF, tissue factor) and reduces the secretion of antithrombotic factors (NO), thereby augmenting this procoagulant state. Interestingly, Lim et al. demonstrated that in patients undergoing AF ablation, both AF induction and rapid atrial pacing were sufficient to cause an increase in the procoagulant molecules P-selectin and thrombin-antithrombin complex after just 15 min. However, only AF induction caused an increase in ADMA levels suggestive of endothelial dysfunction. This study supports the view that a procoagulant state can occur very rapidly following AF induction and that endothelial dysfunction is related to the AF rhythm rather than rapid heart rates.

Finally, endothelial dysfunction affects the atrial endocardium to create a prothrombotic surface (Figure 2C). As previously discussed, endothelial dysfunction is associated with major atrial endocardial histological changes, including microscopic mural thrombi, desquamation, and subendothelial edema and fibrosis.

Noninvasive measures of endothelial function have shown promise as a predictor of adverse outcomes in AF. Raised VWF levels are a predictor of MACES and all-cause mortality, but not stroke. Raised ADMA levels positively correlate with the CHA2DS2-VASc score and are strongly associated with increased stroke and mortality in AF. Lower brachial FMD levels are strongly associated with adverse cardiovascular events, including stroke. In a non-AF cohort, a low RH-PAT index significantly increased the risk of ischemic stroke and improved discrimination of stroke risk by CHA2DS2-VASc score. Assessment of endothelial function may, therefore, play an increasingly important role in AF risk stratification in the future.

5 | DOES AF TREATMENT AFFECT ENDOTHELIAL FUNCTION?

There is strong evidence that restoration of sinus rhythm in AF improves endothelial function. Following electrical cardioversion of AF, studies have shown improvement in endothelial function biomarkers (decreased VWF levels and increased plasma NOX levels), brachial FMD, and hyperemic forearm blood flow induced by acetylcholine or exercise. Similarly, an increase in RH-PAT index is seen up to 6 months following AF catheter ablation. Interestingly, Okawa et al. noted that catheter ablation of persistent, but not paroxysmal AF, was associated with improvements in the RH-PAT index. Endothelial dysfunction also predicts AF recurrence following ablation. Elevated biomarkers of endothelial dysfunction (increased ADMA levels, low baseline brachial FMD, and low RH-PAT index) have all been shown to predict AF recurrence. It is unclear whether antiarrhythmic therapy or rate control drugs are also associated with improvements in endothelial function.
ENDOTHELIAL DYSFUNCTION—A PROMISING NEW THERAPEUTIC PARADIGM?

Although contemporary AF pharmacotherapy relies on rate and/or rhythm control drugs, there is a growing interest in targeting upstream pathways and cardiovascular comorbidity to modify the underlying processes of atrial remodeling and arrhythmic substrate. Although these approaches have largely focused on targeting atrial fibrosis and inflammation, there is emerging evidence that endothelial dysfunction is a promising new addition to this list. Angiotensin-converting enzyme inhibitors (ACE inhibitors)/angiotensin receptor blockers (ARBs), mineralocorticoid receptor blockers (MRAs), and antioxidants (such as N-acetylcysteine and ascorbic acid) have all been shown to reduce the risk of new-onset AF and AF recurrence in large meta-analyses. Statins reduce all-cause and cardiovascular mortality in AF, despite no consistent reduction in AF burden or recurrence. Although these drugs have a multitude of physiological effects, they have all been shown to improve endothelial function. Further studies are required to develop and evaluate the effectiveness of drugs that specifically target endothelial function. Phosphodiesterase-5 (PDE-5) inhibitors improve endothelial function by augmenting cyclic guanosine monophosphate-mediated vasodilatation and upregulating eNOS expression. PDE-5 inhibition has been shown to improve contractile performance and exercise capacity in heart failure with reduced ejection fraction and protect against ventricular arrhythmias in animal models of ischemia–reperfusion injury. Although its early preclinical stage of development, the eNOS transcription enhancer AVE3085 has been shown to restore endothelial function in rat hypertensive and heart failure models. These drugs remain to be investigated in preclinical and clinical studies of AF. It is important to note that endothelial dysfunction is more advanced in persistent and permanent AF compared to paroxysmal AF, and, therefore, treatments aimed at restoring endothelial function may have variable efficacy in these different patient groups.

CONCLUSION

Although progress has been made in understanding the role of endothelial function in AF, significant questions remain unanswered. Several mechanisms coupling endothelial dysfunction to the atrial arrhythmic substrate have been proposed (endothelial–mesenchymal transition and fibrosis, immune cell infiltration and inflammation, and the generation of proarrhythmic ROS) but their effects on the electrophysiological properties of the heart and AF initiation and maintenance remain poorly understood. Noninvasive assessment of endothelial function has been shown to identify patients at high risk of adverse outcome and AF recurrence, but it remains unclear whether these patients benefit from more aggressive treatment of cardiovascular risk factors. Finally, endothelial dysfunction represents a promising upstream target for AF drug therapy. Although several classes of drugs have shown promising results (ACE inhibitors/ARBs, MRAs, antioxidants, and statins), further studies are required to identify and test drugs that specifically target endothelial function.

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