Utilizing biomarkers associated with cardiovascular events in atrial fibrillation: informing a precision medicine response

Nicola Tidbury1 PhD

Joshua Preston1 BSc

Wern Yew Ding1 MRCP

José Miguel Rivera-Caravaca1,2 RN, PhD

Francisco Marín1,2 MD, PhD

Gregory Y. H. Lip1,3 MD

1Liverpool Centre for Cardiovascular Science, University of Liverpool and Liverpool Heart & Chest Hospital, Liverpool, United Kingdom; 2Department of Cardiology, Hospital Clínico Universitario Virgen de la Arrixaca, University of Murcia, Instituto Murciano de Investigación Biosanitaria (IMIB-Arrixaca), CIBERCV, Murcia, Spain.3Aalborg Thrombosis Research Unit, Department of Clinical Medicine, Aalborg University, Aalborg, Denmark

**Corresponding Author:**

Prof Gregory Y H Lip [gregory.lip@liverpool.ac.uk](mailto:gregory.lip@liverpool.ac.uk)

Full mailing address University of Liverpool

William Henry Duncan Building

6 West Derby Street

Liverpool, L7 8TX

Telephone number 0151 794 9020

# Abstract

Introduction: Atrial fibrillation is the most common sustained cardiac rhythm disorder, which currently affects 1-2% of the global population. Furthermore, the incidence and prevalence of atrial fibrillation is rising. Biomarkers have the potential to improve clinical management of patients and therefore reduce the burden on health systems in the future.

Areas covered: A variety of pathways and mechanisms have been associated with atrial fibrillation. This paper provides an overview of a range of blood-based, imaging and genetic biomarkers that are associated with mechanisms and outcomes in atrial fibrillation and their potential use in a clinical setting.

Expert commentary: Atrial fibrillation is becoming increasingly prevalent. Current biomarkers associated with atrial fibrillation such as those involved in myocardial stress, inflammation, hemostasis and fibrosis do not currently provide much additional practical value beyond recommended scores based only on clinical risk factors.

# 1.0 Introduction

Atrial fibrillation (AF) is the most common sustained cardiac rhythm disorder, affecting 1-2% of the general population. The condition is associated with an increasing global incidence and prevalence, partly related to an ageing population. In 2017, it was estimated that there were 37.6 million individuals worldwide with AF [1]. According to current predictions, the prevalence of AF is estimated at 5.6 to 15.9 million in the United States by 2050 [2,3] and 17.9 million in Europe by 2060 [4] alone. Indeed, adults over 40 years old have a 1 in 4 lifetime risk of developing AF [5,6]. Patients with AF have increased morbidity and hospital admission rates, and suffer from a twofold risk of mortality compared to those without AF [7,8]. Furthermore, AF is associated with other serious complications including a fivefold increased risk of stroke [9] and two- to threefold risk of heart failure [7]. As a result, AF represents a growing problem with increasing burden on healthcare services. Estimates from the United States suggest that the condition is linked to an annual cost of $26 billion [10].

A biomarker as specified by the Food and Drug Administration (FDA) is “a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions” [11]. As such, biomarkers may be derived from a wide range of diagnostic assays including biological fluids, imaging parameters and cardiac electrophysiology. The use of biomarkers in cardiovascular medicine is not a new subject, with examples such as troponin now being used in routine clinical care for assessment of myocardial ischaemia. AF is another disease that has received much attention in terms of biomarkers, especially in refining stroke and bleeding risk stratification. Biomarkers associated with inflammation, coagulation, endothelial dysfunction, myocardial stress and injury, and structural remodeling have all been strongly associated with adverse clinical events related to AF. Furthermore, biomarkers provide useful insight into the pathophysiological mechanisms underpinning AF development and progression (figure 1).

In this review, we aim to provide an overview of the range of blood-based, imaging and genetic biomarkers that are associated with mechanisms and outcomes in AF and their potential use in a clinical setting.

# 2.0 Blood-based biomarkers in AF

Cardiac blood-based biomarkers can be broadly divided into the following categories: myocardial stress and injury, inflammatory, thrombotic and fibrotic, identifying the major mechanisms underlying the pathophysiology of AF. A summary of the relevant studies of blood-based biomarkers in AF can be found in Table 1.

## 2.1 Myocardial stress and injury

The troponins and natriuretic peptides are well validated biomarkers of cardiac disease and are widely used for the evaluation of myocardial ischaemia and heart failure, respectively. Given that both these conditions are risk factors for incident AF, serum troponin and plasma natriuretic peptides have also been investigated in AF.

In the normal heart, the natriuretic peptides are secreted in reponse to increased pressure and play a role in the regulation of renal hemodynamics and sodium excretion [12,13]. Natriuretic peptides are commonly released from the ventricles during heart failure but are also thought to be produced in the atria in AF. The latter may be related to the degree of atrial stretch [14]. Furthermore, they have been shown to be elevated prior to the onset of AF diagnosis [15] and increased concentrations of the natriuretic peptides rapidly return to normal following restoration of sinus rhythm [16,17], supporting their role in AF.

The cardiac troponins are regulatory proteins that control the calcium-mediated interaction between actin and myosin. They are mostly bound as a troponin complex, consisting of troponin I, troponin T and troponin C, to cardiac myofibrils but a small pool of free troponins exist in the cytoplasm [18]. During ischemia, necrotic cells release this cytosolic pool of free troponins, followed by the bound proteins as products of degradation [19].

In a sub-study of the RE-LY trial, troponin I and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are both commonly elevated in patients with AF and are associated with an increased risk of stroke, systemic embolism, myocardial infarction and mortality [20]. This was supported by a study of troponin T, in which an increase in troponin T concentrations was associated with a 2.2-fold risk of cardiovascular events in AF patients [21]. A study by Van den Bos *et al.* showed that even minor elevations of troponin I above 0.15 ng/ml in patients with AF, was independently associated with a 2.4-fold increase in all-cause death [22]An increase in all-cause death with increased troponin I was also demonstrated by Kim *et al.* who followed patients with new-onset AF. They also reported that those with troponin I in the highest quartile were most likely to present with persistent AF at their last follow-up visit, suggesting that this biomarker may also be used to identify patients at risk of AF progression [23].

In the ARISTOTLE trial it was found that AF patients with NT-proBNP in the highest quartile had a 2.4-fold risk of stroke or systemic embolism, and 2.5-fold risk of cardiac mortality [24]. Another study by Roldan *et al*., found NT-proBNP was associated with a 2.7-fold risk of stroke and 1.9-fold risk of an adverse cardiovascular event in AF patients [25]. Recently, a small study investigated the use of NT-proBNP as a screening tool for the identification of patients with paroxysmal AF. The authors determined that NT-proBNP concentrations above 95pg/ml were an independent predictor of AF, even among patients who had AF that was only detectable by continuous Holter monitoring rather than a single electrocardiogram (ECG) [26]. Although NT-proBNP may be able to detect ‘hidden’ AF, further studies are needed to confirm its role as a screening tool.

The mechanism(s) relating to the increase in troponins and natriuretic peptides as prognostic tools in AF remain unclear. It is not known whether natriuretic peptides are involved in the pathophysiology of AF or if they are a response to arrhythmia, with any elevated concentrations observed before AF diagnosis being related to undiagnosed paroxysmal AF. Multiple causes of troponin releases have been identified. For example, in patients presenting with AF in an acute setting, troponin concentrations were strongly related to tachycardia and/or angine pectoris [27]. Overall whether tachycardia or other mechanisms underpin the release of troponin in patients with AF is still to be determined.

## 2.2 Inflammatory markers

Inflammation has long been associated with a wide variety of cardiovascular conditions from which AF is no exception. Inflammation is thought to play an important role in structural and electrical remodeling of the atria, therefore contributing to AF development and persistence. Various inflammatory markers such as C-reactive protein (CRP) [28–30] and pro-inflammatory interleukins [21,31,32], such as interleukin-6 (IL-6), have been associated with the presence and outcomes of AF.

C-reactive protein is a non-specific biomarker that is routinely measured to assess levels of inflammation and may be used to provide an indication of the risk of cardiac disease. The use of CRP has been investigated in a range of scenarios from predicting the future incidence of AF [29] to determining the risk of AF recurrenceafter treatment [33,34]. Another commonly investigated inflammatory marker is IL-6, an inflammatory cytokine, which induces the synthesis of CRP in the liver.

Chung *et al.* demonstrated that there was a twofold increase in CRP among patients diagnosed with AF, compared to those without a previous AF diagnosis. Furthermore, subgroup analysis demonstrated that those with persistent AF had higher CRP levels than those with paroxysmal AF. There was also an elevation in CRP concentrations among AF patients who had blood samples collected during periods of AF compared to those who were in sinus rhythm (SR) at the time [29]. Although the latter observation was confirmed in a study by Marcus *et al.*, the authors failed to detect any significant difference in CRP levels among patients who were in SR, regardless of whether they had known AF previously [35]. Marcus *et al.* also investigated the intra- and extra-cardiac concentrations of both high sensitivity CRP (hsCRP) and IL-6, and found that AF results in the sequestration of inflammatory cytokines in the heart.

C-reactive protein has also been investigated as a possible predictor of outcomes following treatment in AF. Several studies have demonstrated that high levels of hsCRP increased the risk of AF recurrence following electrical cardioversion [33,34]. In a longitudinal study by Kallergis *et al.*, restoration and maintenance of SR resulted in a gradual decrease of hsCRP, with concentrations of CRP significantly lower than baseline at one month after cardioversion. As such, the authors concluded that inflammation may be a consequence rather than a cause of AF [34]. In contrast, a separate study showed that patients with lone AF had comparable CRP levels to controls [36]. However, patients with concomitant cardiac disease had significantly increased concentrations of CRP, resulting in the conclusion that underlying conditions are the cause of inflammation and not AF itself [36]. As CRP is a marker of general inflammation, it is likely that its clinical use in AF is limited. Patients with AF often have several co-morbidities, many of which have an inflammatory element which can increase CRP concentrations.

A study by Conway *et al.* highlighted the potential pro-thrombotic effects of IL-6, with increased concentrations conferring a 2.9-fold risk of stroke [37]. This association was supported in a larger cohort from the RE-LY trial [38]. The findings highlight the potential role of inflammation in the prothrombotic state of AF. Interleukin-6 enhances platelet generation, and increases sensitivity to thrombin and transcription of fibrinogen. Furthermore, IL-6 is also associated with endothelial activation and damage [39,40]. It has also been independently linked to concentrations of tissue factor and an increased stroke risk [31].

White blood cells (WBCs) have also been linked to AF. In the Framingham Heart Study, an increased WBC count was associated with incident AF during 5 years of follow-up [41]. In addition, WBC count has been shown to decrease following successful cardioversion of patients with AF [42]. A meta-analysis of hemotological parameters also showed that WBC count is related to AF recurrence but not to the presence of AF [43]. Individual subsets of WBCs have also been linked to AF. For example, the intermediate (CD14++ CD16+) sub-population of monocytes has been associated with the presence AF [44]. However, similar to CRP, WBCs are increased in many other disease states limiting their prognostic value in AF.

Despite the evidence linking inflammatory markers to AF, the use of them clinically as prognostic markers require further evidence. Given the non-specific nature of inflammatory biomarkers, it should generally be used only to provide a general overview of the underlying health of a patient and their comorbidities.

## 2.3 Markers of thrombosis

The complications of AF include systemic thromboembolism and stroke, and there is evidence to support a pro-thrombotic or hypercoagulable state among these patients. Atrial fibrillation fulfils the requirements for Virchow’s triad for thrombogenesis, namely abnormal blood flow, vessel wall and blood constituents. Therefore several biomarkers relating to the thrombotic state have been investigated in AF cohorts.

D-dimer is a small protein fragment that is released following fibrinolysis that is clinically used to exclude thromboembolic events. Nozawa *et al.* demonstrated that patients with non-valvular AF and D-dimer levels <150ng/ml were at a significantly lower risk of thromboembolic events (0.7% per year vs 3.8% per year) than patients with concentrations ≥150ng/ml [45]. Similar findings have been reported in other studies [46,47]. However, You *et al.* concluded that baseline D-dimer concentration was not an independent risk factor for stroke in AF patients [48]. Overall, it is likely that D-dimer has some prognostic value for stroke risk stratification. However, further investigation is warranted to evaluate its role in this area.

Similarly, soluble fibrin monomer complex (SFMC) is a biomarker of fibrin formation that is abnormally elevated in the context to hypercoagulability. In AF patients, high SFMC levels have been shown to be associated with the risk of adverse cardiovascular events, cardiovascular mortality and all-cause mortality [49,50].

Von Willebrand Factor (vWF), a widely recognized marker of endothelial damage/dysfunction, is another biomarker that has been extensively studied in AF. It is a crucial component in hemostasis, mediating adhesion at sites of vascular injury and promoting platelet aggregation during high shear stress. Von Willebrand Factor has been shown to be elevated in AF patients among whom it is positively correlation to an increased risk of cardiovascular events [51–53]. The Rotterdam study demonstrated a positive association of vWF concentration with AF incidence, particularly in females, perhaps explaining their increased stroke risk compared to males [54]. Increased vWF levels were also independently correlated to incidence of left atrial appendage (LAA) thrombus in AF [55], a key location for clot formation in patients with AF [56]. In contrast, Ehrlich *et al.* found that there was no association between vWF and a composite of cardiovascular endpoints (myocardial infarction, stroke and systemic embolism) [57]. Despite much evidence for an association between vWF and thrombus risk, it is unlikely to find a clinical application in AF due to its poor specificity, as concentrations are also increased in multiple other disorders.

The prothrombotic state in AF has led to the publication of numerous studies on the role of platelets but the results have been contradictory. Whilst changes to platelets do exist in AF patients, their correlation to the risk of thromboembolism remains unclear. Recently a post-hoc analysis of patients in a Japanese registry, found that platelet count had no impact on outcomes in AF patients [58]. In contrast, a prospective registry study comparing patients with non-valvular AF with either thrombocytopenia or abnormal platelet count, found those with thrombocytopenia had a significantly lower stroke risk [59].

Platelet activation has also been extensively studied. Choudhury *et al.* reported that whilst AF patients had increased levels of soluble P-selectin, a marker of platelet activation, and platelet microparticles compared with healthy controls in SR, there were no differences to disease matched controls. The authors therefore concluded that elevations of platelet activation in AF are the consequence of underlying comorbidities rather than the arrhythmia *per se* [60]. Another marker of platelet activation, β-thromboglobulin, has been found to be increased in patients with AF compared to controls in SR [61–63]. However, despite the presence of elevated platelet activation in AF, there is little evidence that it relates directly to an elevated thromboembolic risk. This is supported by a sub-study of the SPAF-III trial which showed no correlation between β-thromboglobulin levels and subsequent thromboembolic events [64]. As such, there is limited evidence for the use of platelet counts and platelet activation to inform clinical decision-making in AF.

## 2.4 Atrial fibrosis markers

Biomarkers of fibrosis are possibly some of the most promising in AF and have the potential of added value to clinical assessments of AF patients. The involvement of fibrosis and structural remodeling as underlying pathologies of cardiac diseases are becoming increasingly recognised. In AF, fibrosis and structural remodeling of the atria are thought to drive the disease progression from paroxysmal to sustained AF. This is a complex process involving a vast array of biological pathways and potential biomarkers, a handful of which are discussed below.

Transforming growth factor - beta 1 (TGF-β1) is a pro-fibrotic cytokine that is heavily involved in the regulation of key process including immune function, cell proliferation, cell migration, apoptosis and fibrosis [65,66]. It has also been implicated in the pathologies of many cardiovascular diseases such as hypertension, re-stenosis and heart failure due to its role in cardiac fibrosis [67]. Although it could be argued that TGF-β1 is an inflammatory marker, it has a major role in fibrosis by promoting collagen synthesis within cardiac fibroblasts and upregulating the expression of collagen genes via the SMAD signaling pathway [68–70].

A meta-analysis of found that elevated levels of TGF-β1 concentrations are associated with an increased risk of new onset AF [71]. A study by Lin *et al.* also found higher concentrations of TGF-β1 in patients with sustained AF compared to a SR cohort [72]. Furthermore, TGF-β is upregulated on CD14+ monocytes in patients with AF and severe fibrosis [73]. It has also been found to be an independent predictor of AF recurrence after catheter AF ablation [74]. Although TGF-β1 has been identified as a possible marker of AF outcomes and progression,it is expressed in a variety of tissues and is upregulated in many diseases, therefore it is unlikely to be specific enough as a biomarker of AF.

Growth-differentiation factor 15 (GDF-15) is another member of the TGF-β superfamily and is a stress response cytokine. It utilizes the same receptors as TGF-β1 [75] to activate the p53 pathway [76]. In AF, GDF-15 was mainly associated with an increased risk of bleeding and mortality [77–79]. The association of this biomarker with stroke risk remains under debate. Hu *et al.* found that levels of GDF-15 were associated with a significantly increased risk of left atrial (LA)/LAA thrombus [80]. However, large cohorts in sub-studies of the RE-LY and ARISTOTLE trials did not demonstrate any association between GDF-15 and stroke risk, after adjustment for other biomarkers [81,82].

Galectin-3 (Gal-3) plays an important role in the regulation of tissue fibrosis, angiogenesis and inflammatory pathways [83]. Elevated Gal-3 synthesis has been implicated in a number of fibrotic disease pathologies such as lung fibrosis and heart failure [84]. This biomarker has recently been implicated as a cause of fibrosis in AF. Concentrations of Gal-3 increase with AF disease burden such that patients with persistent AF have significantly higher levels of Gal-3 than patients with paroxysmal AF [85,86]. Furthermore, a recent meta-analysis found that patients with higher Gal-3 levels had a 45% increase in the odds of developing AF [87]. Galectin-3 has also been identified as an independent predictor of the recurrence of AF following catheter ablation [86].

Soluble suppression of tumorigenicity 2 (sST2) is a cardiac biomarker that is upregulated in cardiomyocytes following a stress response [88]. Higher ST2 levels have been identified in patients with heart failure and have frequently been associated with an increase in mortality [89,90]. In the Framingham Heart Study, sST2 was unable to improve AF risk discrimination for incident AF [91]. However, a more promising a study by Vilchez *et al.* found that sST2 levels were an independent predictor of all-cause mortality in anti-coagulated AF patients and could be used to improve clinical risk assessment in this cohort [92]. Elevated sST2 levels may also be a useful screening tool following catheter ablation, where they may signify increased levels of atrial fibrosis that are associated with an early recurrence of AF [93,94]. Therefore, sST2 may be useful to determine the effectiveness of treatment in AF.

Metalloproteinases (MMPs) are heavily involved in the modulation of extra-cellular matrix (ECM) turnover through the degredation of its components. The different subgroups of MMPs have different substrate specificity due to slight structural differences [95]. MMPs have been subjected to numerous studies in order to determine their level of association with AF and its associated remodeling. A study by Li *et al*., found that MMP-9 concentration increases with progression of the atrial fibrillation, therefore implicating MMP-9 with structural remodeling observed in AF [96]. Another study that investigated the tissue concentrations of MMP-9 in patients with and without AF that underwent cardiac surgery, also found that AF patients had a increased of MMP-9. Immunohistochemistry analysis of the samples determined that the increase of MMP-9 predominantly took place within the perivascular regions of the atrium [97]. MMP-2 has also been indicated in structural remodeling of the left atrium and is associated with an enlarged left atrium volume [98]. MMP2 and tissue inhibitor of metalloproteinase inhibitor (TIMP)-2 have also been investigated in recurrence of AF following catheter ablation. This showed that MMP2 levels slowly increased in the 12 months following the procedure, indicating tissue remodeling. However, it was found that TIMP-2 levels were also increased in those with no occurrence of AF, whereas TIMP-2 were more likely to remain stable in the recurrence group [99]. A higher TIMP-1/MMP-1 ratio has also been observed in those patients that did not develop post-operative AF [100]. In contrast, Marin *et al*., found there was no association between AF incidence and MMP-1 [101], an observation seen elsewhere [102], but did observe an association between MMP-1/TIMP-1 and a pro-thrombotic state.

In summary, in the current state there is not evidence for the use of biomarkers of atrial fibrosis and structural remodeling in the clinic. However, a greater emphasis is being placed on the investigation of markers of fibrosis and structural remodeling in cardiac disease. In AF, newer atrial fibrosis markers, such as Gal-3, look promising for predicting early recurrence following catheter ablation, possibly reflecting greater changes in the left atrium as AF progresses.

## 2.5 Risk scores

The CHA2DS2-VASc score is among the most widely employed scoring system in AF that is used for thromboembolic risk stratification to aid decisions on anti-coagulation therapies [103]. However, despite being considered the best clinical tool in this regard, it only has modest prognostic value [104]. As such, several studies have attempted to refine this clinical score by the addition of blood-based biomarkers.

The first study using biomarkers to refine clinical risk stratification was published over 10 years ago, where vWF was able to improve on risk prediction over the CHADS2 score and Birmingham schema (the precursor to CHA2DS2-VASc score) [105]. Since then other biomarkers such as NT-proBNP [24], IL-6 [38] and vWF [51,52] have all been shown to increase the predictive capability of the CHA2DS2-VASc score. Despite this, the overall prognostic value of the modified tool remained modest at best. Indeed, a study by Rivera-Caravaca *et al.* determined that despite elevated concentrations of vWF, troponin T, IL-6 and NT-proBNP in AF patients with a CHA2DS2-VASc score ≥2, inclusion of these biomarkers either individually or as a whole did not significantly increase the predictive performance of the CHA2DS2-VASc score [53]. Another study by Roldan, determined that the same biomakers improved the overall predictive value of the CHA2DS2-VASc score, however the predictive value remained modest and the net benefit over the current clinical scores remained lower [106].

There has been the development of biomarker-based risk scores to try and improve risk prediction in AF, such as the ABC (Age, Biomarkers (NT-proBNP and troponin), and Clinical history of stroke/TIA) stroke score [107]. Several sub-studies of clinical trials using the ABC stroke score found that it significantly improved the prediction of stroke risk with c-indices of 0.65-0.68 vs 0.6-0.62 for the CHA2DS2-VASc score [107–109]. However, in real-world cohorts of patients, the ABC stroke score and the addition of other biomarkers did not perform any better than the CHA2DS2-VASc score, especially during long-term follow-up [110].

The HAS-BLED score is used in conjunction with the CHA2DS2-VASc score to determine bleeding risk in AF patients [111]. An ABC-bleeding score (Age, Biomarkers [GDF-15, troponin, haemoglobin] and Clinical risk of bleeding) has also been developed in an attempt to use biomarkers to improve bleeding risk stratification in AF [112]. The ABC-bleeding risk score performed better in clinical trial cohorts than the HAS-BLED score [112]. However, when validated in real-world cohorts, the ABC-bleeding score did not have any added advantage over the HAS-BLED score [113]. The HAS-BLED score provides then the best prediction for bleeding risk [104].

In the same vein as the other ABC scores, an ABC-death score, to predict mortality, has also been developed based on age, NT-proBNP, troponin T, GDF-15 and heart failure. It demonstrated good c-indices in both derivation and validation cohorts (0.74) [114] and may contribute to overall risk assessment in AF.

However, non-specificity of biomarkers in AF remains a frequent disadvantage, as they predict ‘sick’ patients or poor prognosis overall. Recently, the ABC scores (both, ABC-stroke and ABC-bleeding) demonstrated similar predictive ability for outcomes beyond stroke and bleeding, including myocardial infarction, heart failure, cardiovascular events, and all-cause deaths in AF patients [115]. Indeed, several questions regarding biomarkers remain unanswered. Many of the common biomarkers have been derived from highly selected randomized clinical trial (RCT) cohorts, with only a single measurement beingassociated with the desired endpoints at a much later time. Nevertheless, ‘real world’ patients are different from RCT patients [116], and risk is not a static process since patients get older and develop incident risk factors [117–119]. Additionally, some biomarkers have a diurnal variation, and inter- and intra-patient variability. Furthermore, limited access to laboratories in different health care systems may hinder the use of these biomarkers [120,121]. Hence, application of biomarkers in clinical practice goes beyond an assessment of “statistical significance” and should take into consideration issues such as practicality and costs.

# 3.0 Imaging biomarkers in AF

A variety of imaging techniques may be used to assess structural changes that occur in AF, particularly within the LA. To this end, echocardiography, computed tomography (CT) and magnetic resonance imaging (MRI) techniques have all been employed to investigate AF with a focus on LA parameters (table 2).

## 3.1 LA size and function

Left atrial volume and function have been thoroughly investigated in AF patients. A sub-study of the ENGAGE AF-TIMI 48 trial measured LA volume and function in 971 patients with a history of AF. The authors found that LA enlargement was present in nearly two-thirds of all patients and the percentage of the cohort with LA enlargement increased with AF burden such that 48% of patients with paroxysmal AF had LA enlargement and this increased to 77% in patients with permanent AF. Left atrial function, as measured by left atrial ejection fraction (LAEF), measured using transthoracic echocardiography (TTE), also declined across the spectrum of AF types [122]. The association between LA volume and/or LA function with AF has also been observed in other studies [123,124].

Furthermore, LA volume and function have also been associated with the incidence of stroke in AF. A study by Dakay *et al.* found that AF patients who suffered a stroke despite appropriate anti-coagulation were more likely to have moderate to severe enlargement of the LA [125]. The finding is supported by a study of the Fushimi AF registry in which LA enlargement was an independent predictor of stroke or systemic embolism [124].

Left atrial size and function have also been associated with AF recurrence following catheter ablation. Indeed, a meta-analysis found that elevated LA diameter values were significantly associated with AF recurrence in patients following catheter ablation [126]. A recent study by Oka *et al*. also found that LAEF was a useful marker for the recurrence of AF after catheter ablation [127].

Furthermore, newer methods such as speckle-tracking echocardiography (STE), look promising in AF, using left atrial strain as a measurement of atrial function. A decreased left atrial strain rate has been associated with stroke in patients with AF [128,129], and addition of LA strain and LA volume to the CHADS2 score has been shown to improve the prediction of hospitalization or death from cardiovascular events [130]. Other measurements of the LA using STE have also been found to be associated to stroke risk in AF patients [131,132].

Therefore, LA size and function may be used to improve risk stratification in AF. An advantage of these biomarkers are that they can be assessed using relatively simple and routine tranthoracic echocardiography techniques.

## 3.2 LAA thrombus and morphology

The LAA is a remnant of the embryonic LA and is a key site for thrombus formation in AF patients [56]. It has a long, complex structure with a narrow inlet which provides ideal conditions for blood stasis and therefore thrombus formation. The thrombus may then dislodge causing a stroke or other thromboembolic events. A pooled study of AF patients who underwent cardiac surgery, trans-esophageal echocardiogram (TEE) or an autopsy showed that 14% had thrombi in the LA, with the majority of them originating from the LAA [133]. Another study which included 137 patients with a recent thromboembolic event who underwent TEE found that approximately one-fifth had evidence of an LAA thrombus [134].

The LAA morphology has also been linked to stroke risk in AF patients. A study by Di Biase *et al.* categorised AF patients according to the appearance of the LAA on cardiac imaging. They split patients into four main groups: chicken wing (48%), cactus (30%), windsock (19%) and cauliflower (3%). These different morphologies were associated with varying levels of stroke risk. Chicken wing morphology was associated with the lowest risk of stroke, followed by windsock and cactus morphologies with a 4-fold increase in stroke risk. Lastly patients with a cauliflower morphology had an 8-fold increased risk of stroke when compared with the chicken wing reference group [135]. Similar findings have also been reported elsewhere [136,137]. However, Khurram *et al.* found no association between LAA morphology and the risk of stroke. Furthermore, the authors reported significant inter-observer variability suggesting that this may be an unreliable method of assessment [138]. Further investigations would be required to determine whether the utilization of LAA morphology in stroke risk stratification, in a general cohort of AF patients, is clinically relevant.

## 3.3 Atrial fibrosis using LGE-MRI

In addition to blood-based biomarkers, myocardial fibrosis can be directly identified using late gadolinium enhancement (LGE)-MRI. The contrast agent, gadolinium, is taken up and quickly returned to the blood in healthy tissue, whereas it is retained and returned at a much slower rate in fibrotic tissue with a large extracellular space. The degree of fibrosis is often evaluated using the Utah classification of AF. Patients are classified to one of four categories based on the percentage of fibrosis, with Utah IV being patients with the highest degree of fibrosis [139].

Late gadolinium enhancement MRI (LGE-MRI) has been used in several studies to establish the pattern of atrial fibrosis, recurrence of AF after catheter ablation, and stroke risk. In AF, there appears to be a preferential accumulation of fibrosis around the antrum of the left inferior pulmonary vein [140,141]. Fibrosis has also been observed in the LAA. Akoum *et al.*demonstrated that LAA fibrosis, as detected by LGE-MRI, was associated with reduced flow velocities and independently associated with LAA thrombus [142].

Many studies have found that increased levels of atrial fibrosis are associated with AF recurrence post-ablation [143,144]. Khurrum *et al*. determined that advanced stages of fibrosis with LGE> 35% (Utah IV) are associated with poor outcomes for ablation [145]. In contrast, Sramko *et al.* found that the extent of atrial fibrosis determined by LGE-MRI prior to ablation does not predict AF recurrence [146]. These differences may be accounted for by the fact that assessment of atrial fibrosis using LGE-MRI does not adhere to a standardised protocol, complicating the validation of this as a useful biomarker. Despite the possible value of LGE-MRI to determine the role of catheter ablation in selected patients, it is hampered from routine use by limited availability, and high associated costs and expertise to perform the test and interpret the findings.

# 4.0 Genetic biomarkers

Research into the genetics of AF has found at least 138 common genetic variants associated with the condition [147]. Whilst a comprehensive review of the genetics of AF is outside the scope of this article, Roselli *et al* [147], Feghaly *et al* [148] and Shoemaker *et al* [149] all provide a greater depth analysis of the topic. The genetic predisposition of AF is not a new concept. Several studies have described familial clustering of AF in a large population. Fox *et al*. identified through the Framingham Heart Study that a third of patients diagnosed with AF also had at least one parent with a similar diagnosis [150]. This is supported by a genetic study of over 5,000 people, in Iceland,where the relative risk of developing AF was 1.77, if a first degree family member had also received an AF diagnosis [151]. Lone AF, in particular, seems to have a particularly strong genetic basis [150,152]. In situations where there is a strong familial link of AF, researchers have identified mutations in several ion channels [153–158]. For example, a serine to glycine missense mutation at position 140 (S140G) of KCNQ1 gene has been found in a family of Chinese descent [159]. This results in a gain of function in potassium channels that cause the slowly repolarizing current. This is predicted to shorten the action potential, thereby predisposing to re-entrant circuits and subsequent AF [160]. However, it should be acknowledged that overall genetic mutations are a rare cause of AF [161].

Genome wide association studies have identifiedan association between the 4q25 locus and AF. This is perhaps one of the most researched genetic causes for AF [162–164]. The most significant variation is located in an intergenic region that is located 150 kilobases from the PITX2 gene. PITX2 is involved during embryonic development and is essential for development of the sinus node, formation of the atria and ensuring physiological left-right heart asymmetry [162,163]. Another locus that has been shown to be associated with AF is ZFHX3, which encodes a transcription factor that is enriched in cardiac tissue [165,166], and interacts with PITX2 [167].

Genetic variants of the 4q25 locus have also been associated with stroke risk in AF [168,169] and there have been investigations into polymorphisms into various proteins that may be associated with thrombosis and hemostasis. Factor XIII Val134Leu polymorphism has been independently associated with IL-6 levels in AF which may influence the prothrombotic state [170]. The β-fibrinogen gene 455 G/A polymorphism has also been associated with cardioembolic stroke, potentially through elevated fibrinogen levels [171]. Another study found factor VII insertion at position 323 has been associated with lower plasma F1+2 levels and lower stroke risk whilst an 807C/T polymorphism of glycoprotein 1a was associated with a higher stroke risk in AF patients [172]. However, Factor V Leiden mutation, which can increase the chance of developing thrombosis, has not been found to be predictive of thromboembolism in AF [173].

In addition, the role of genetic markers in the risk of adverse events should also be mentioned. For example, rs2431697 of miR-146a, a negative regulator of inflammation, could help in the prediction of cardiovascular events [174] and neutrophil extracellular traps aid in prognostic information [175].

There have been great advances in the genetic mapping of AF. However, several mutations that have been identified in family clusters are often unique and we do not yet understand the mechanism of action of chromosome 4 variants, there is also not enough evidence to support the role of genetic biomarkers in stroke risk in AF. Despite the progress made, the potential of genetics in AF risk stratification remains to be unlocked.

# 5.0 Conclusion

Multiple biomarkers have been associated with AF including progression of the disease and long-term outcomes, however as yet the inclusion of biomarkers have found limited success in the clinic due to issues with specificity and practicality of use.

# 6.0 Expert Opinion

The global incidence of atrial fibrillation (AF) is rising due to an ageing population, with multiple comorbidities. AF is a multifactorial disease associated with several cardiovascular events, including thromboembolism and heart failure; hence a move towards a more holistic or integrated approach that includes proactive management of pre-existing comorbidities [176]. The mechanisms which underly the development and progression of AF are not fully understood. Many cardiac-related biomarkers of myocardial stress, inflammation, hemostasis and fibrosis have been studied in AF, as summarized in table 3. However, the majority reflect underlying comorbidities rather than AF *per se*. Furthermore, ageing and incident comorbidities may affect stroke and bleeding risks, and risk assessment should be a dynamic process (and not a static ‘one-off’ assessment) [118].

The current mainstay of AF management includes mitigation of thromboembolic risk using anti-coagulation therapy; however, the latter is associated with an increased risk of bleeding. Therefore, it is important to identify AF patients who may derive a net benefit from anticoagulation, whilst balancing simplicity, practicality and usefulness, of any tools used, in daily clinical practice, as well as considering therapy persistence [119,177,178]. Unfortunately, the addition of biomarkers to current thromboembolic risk stratification tools in AF fails to provide significant clinical value beyond the already recommended CHA2DS2-VASc score [53,110] and factors such as practicality of use, assay availability and variability further hamper their clinical use. To aid clinical decisions, bleeding risk scores have also been developed. The addition of biomarkers in these tools were promising when evaluated in clinical trials but failed to perform much better in real-world cohorts. This highlights the problem of transferring promising biomarkers from a clinical trial into the real-world environment where patients may be older, have multiple co-morbidities and have a greater propensity for polypharmacy [120].

Some evidence suggests that blood-based and imaging biomarkers of atrial fibrosis may help to identify patients at risk of early recurrence following catheter AF ablation. Newer blood based biomarkers of atrial fibrosis, such as galectin-3, and commonly used imaging techniques, such as transthoracic echocardiography, may be able to help clinicians and patients by providing increased information to make an informed decision about their treatment. However, the utility of newer techniques in this area, such as late gadolinium enhanced (LGE)-MRI to establish the level of atrial fibrosis, are hampered by their availability, associated costs and required expertise to analyze the data. Future advances in technology may help promote these as viable options to assist in the determination of the best clinical course for AF treatment.

Research into the role of genetics in AF is also accelerating at a rapid pace, with hundreds of mutations and gene variants now associated with AF risk. Although a definitive gene has yet to be established in the wider population, studies in the area of genetics may incidentally provide us with new mechanisms or proteins to research. As with several other diseases, microRNAs are increasingly being explored to ascertain their clinical value, and AF is no exception, offering another area of research that warrants further investigation.

Overall, current studies do provide us with a glimmer of hope for precision medicine in the treatment of AF. Biomarkers involved in atrial fibrosis and structural remodeling hold the potential to become valuable clinical biomarkers in the future. Furthermore, newer molecular techniques such as -omics studies, that are not discussed here, may reveal novel biomarkers in these pathways. However, the current biomarkers associated with AF do not provide benefit over more practical and cost-effective clinical risk scores and therefore have little utility in the clinic.

**Declarations of interest**

Gregory Y H Lip declares being a consultant for Bayer/Janssen, BMS/Pfizer, Medtronic, Boehringer Ingelheim, Novartis, Verseon and Daiichi-Sankyo and a speaker for BMS/Pfizer, Medtronic, Boehringer Ingelheim, and Daiichi-Sankyo. No fees are directly received personally.

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

**References**

Papers of special note have been highlighted as: \* of interest \*\* of considerable interest to readers.

[1] Lippi G, Sanchis-Gomar F, Cervellin G. Global epidemiology of atrial fibrillation: An increasing epidemic and public health challenge. Int. J. Stroke. 2020;

[2] Miyasaka Y, Barnes ME, Gersh BJ, et al. Secular trends in incidence of atrial fibrillation in Olmsted County, Minnesota, 1980 to 2000, and implications on the projections for future prevalence. Circulation. 2006;114:119–125.

[3] Go AS, Hylek EM, Phillips KA, et al. Prevalence of diagnosed atrial fibrillation in adults: National implications for rhythm management and stroke prevention: The anticoagulation and risk factors in atrial fibrillation (ATRIA) study. J. Am. Med. Assoc. 2001;285:2370–2375.

[4] Krijthe BP, Kunst A, Benjamin EJ, et al. Projections on the number of individuals with atrial fibrillation in the European Union, from 2000 to 2060. Eur. Heart J. 2013;34:2746–2751.

[5] Lloyd-Jones DM, Wang TJ, Leip EP, et al. Lifetime risk for development of atrial fibrillation: The Framingham Heart Study. Circulation. 2004;110:1042–1046.

[6] Heeringa J, Van Der Kuip DAM, Hofman A, et al. Prevalence, incidence and lifetime risk of atrial fibrillation: The Rotterdam study. Eur. Heart J. 2006;27:949–953.

[7] Wang TJ, Larson MG, Levy D, et al. Temporal relations of atrial fibrillation and congestive heart failure and their joint influence on mortality: The Framingham heart study. Circulation. 2003;107:2920–2925.

[8] Benjamin EJ, Wolf PA, D’Agostino RB, et al. Impact of atrial fibrillation on the risk of death: The Framingham Heart Study. Circulation. 1998;98:946–952.

[9] Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation as an independent risk factor for stroke: The Framingham Study. Stroke. 1991;22:983–988.

[10] Kim MH, Johnston SS, Chu BC, et al. Estimation of total incremental health care costs in patients with atrial fibrillation in the united states. Circ. Cardiovasc. Qual. Outcomes. 2011;4:313–320.

[11] Califf RM. Biomarker definitions and their applications. Exp. Biol. Med. 2018;243:213–221.

[12] La Villa G, Fronzaroli C, Lazzeri C, et al. Cardiovascular and renal effects of low dose brain natriuretic peptide infusion in man. J. Clin. Endocrinol. Metab. 1994;78:1166–1171.

[13] Holmes SJ, Espiner EA, Richards AM, et al. Renal, endocrine, and hemodynamic effects of human brain natriuretic peptide in normal man. J. Clin. Endocrinol. Metab. 1993;76:91–96.

[14] Goetze JP, Friis-Hansen L, Rehfeld JF, et al. Atrial secretion of B-type natriuretic peptide. Eur. Heart J. 2006. p. 1648–1650.

[15] Patton KK, Ellinor PT, Heckbert SR, et al. N-Terminal pro-b-type natriuretic peptide is a major predictor of the development of atrial fibrillation: The cardiovascular health study. Circulation. 2009;120:1768–1774.

[16] Yamada T, Murakami Y, Okada T, et al. Plasma Atrial Natriuretic Peptide and Brain Natriuretic Peptide Levels After Radiofrequency Catheter Ablation of Atrial Fibrillation. Am. J. Cardiol. 2006;97:1741–1744.

[17] Wożakowska-Kapłon B. Effect of sinus rhythm restoration on plasma brain natriuretic peptide in patients with atrial fibrillation. Am. J. Cardiol. 2004;93:1555–1558.

[18] Bleier J, Vorderwinkler K-P, Falkensammer J, et al. Different intracellular compartmentations of cardiac troponins and myosin heavy chains: A causal connection to their different early release after myocardial damage. Clin. Chem. 1998;44:1912–1918.

[19] Collinson PO, Boa FG, Gaze DC. Measurement of Cardiac Troponins. Ann. Clin. Biochem. 2001;38:423–449.

[20] Hijazi Z, Oldgren J, Andersson U, et al. Cardiac biomarkers are associated with an increased risk of stroke and death in patients with atrial fibrillation: A randomized evaluation of long-term anticoagulation therapy (RE-LY) substudy. Circulation. 2012;125:1605–1616.

[21] Roldán V, Marín F, Díaz J, et al. High sensitivity cardiac troponin T and interleukin-6 predict adverse cardiovascular events and mortality in anticoagulated patients with atrial fibrillation. J. Thromb. Haemost. 2012;10:1500–1507.

[22] Van Den Bos EJ, Constantinescu AA, Van Domburg RT, et al. Minor elevations in troponin I are associated with mortality and adverse cardiac events in patients with atrial fibrillation. Eur. Heart J. 2011;32:611–617.

[23] Kim BS, Kwon CH, Chang H, et al. Usefulness of High-Sensitivity Troponin I to Predict Outcome in Patients With Newly Detected Atrial Fibrillation. Am. J. Cardiol. 2020;125:744–750.

[24] Hijazi Z, Wallentin L, Siegbahn A, et al. N-terminal pro-B-type natriuretic peptide for risk assessment in patients with atrial fibrillation: Insights from the ARISTOTLE trial (Apixaban for the prevention of stroke in subjects with atrial fibrillation). J. Am. Coll. Cardiol. 2013;61:2274–2284.

[25] Roldán V, Vílchez JA, Manzano-Fernández S, et al. Usefulness of N- Terminal pro-B- Type natriuretic peptide levels for stroke risk prediction in anticoagulated patients with atrial fibrillation. Stroke. 2014;45:696–701.

[26] Palà E, Bustamante A, Clúa-Espuny JL, et al. N-Terminal Pro B-Type Natriuretic Peptide’s Usefulness for Paroxysmal Atrial Fibrillation Detection Among Populations Carrying Cardiovascular Risk Factors. Front. Neurol. 2019;10.

[27] Parwani AS, Boldt L-H, Huemer M, et al. Atrial fibrillation-induced cardiac troponin I release. Int. J. Cardiol. 2013;168:2734–2737.

[28] Wu N, Xu B, Xiang Y, et al. Association of inflammatory factors with occurrence and recurrence of atrial fibrillation: A meta-analysis. Int. J. Cardiol. 2013;169:62–72.

[29] Chung MK, Martin DO, Sprecher D, et al. C-reactive protein elevation in patients with atrial arrhythmias: Inflammatory mechanisms and persistence of atrial fibrillation. Circulation. 2001;104:2886–2891.

[30] Lee Y, Park H-C, Shin J-H, et al. Single and persistent elevation of C-reactive protein levels and the risk of atrial fibrillation in a general population: The Ansan-Ansung Cohort of the Korean Genome and Epidemiology Study. Int. J. Cardiol. 2019;277:240–246.

[31] Conway DSG, Buggins P, Hughes E, et al. Relationship of interleukin-6 and C-reactive protein to the prothrombotic state in chronic atrial fibrillation. J. Am. Coll. Cardiol. 2004;43:2075–2082.

[32] Liuba I, Ahlmroth H, Jonasson L, et al. Source of inflammatory markers in patients with atrial fibrillation. Europace. 2008. p. 848–853.

[33] Korantzopoulos P, Kalantzi K, Siogas K, et al. Long-term prognostic value of baseline C-reactive protein in predicting recurrence of atrial fibrillation after electrical cardioversion. PACE - Pacing Clin. Electrophysiol. 2008;31:1272–1276.

[34] Kallergis E, Manios EG, Kanoupakis EM, et al. The role of the post-cardioversion time course of hs-CRP levels in clarifying the relationship between inflammation and persistence of atrial fibrillation. Heart. 2008;94:200–204.

[35] Marcus GM, Smith LM, Ordovas K, et al. Intracardiac and extracardiac markers of inflammation during atrial fibrillation. Hear. Rhythm. 2010;7:149–154.

[36] Ellinor PT, Low A, Patton KK, et al. C-Reactive Protein in Lone Atrial Fibrillation. Am. J. Cardiol. 2006;97:1346–1350.

[37] Conway DSG, Buggins P, Hughes E, et al. Prognostic significance of raised plasma levels of interleukin-6 and C-reactive protein in atrial fibrillation. Am. Heart J. 2004;148:462–466.

[38] Aulin J, Siegbahn A, Hijazi Z, et al. Interleukin-6 and C-reactive protein and risk for death and cardiovascular events in patients with atrial fibrillation. Am. Heart J. 2015;170:1151–1160.

[39] Burstein SA, Peng J, Friese P, et al. Cytokine-induced alteration of platelet and hemostatic function. Stem Cells. 1996;14:154–162.

[40] Yudkin JS, Stehouwer CDA, Emeis JJ, et al. C-reactive protein in healthy subjects: Associations with obesity, insulin resistance, and endothelial dysfunction: A potential role for cytokines originating from adipose tissue? Arterioscler. Thromb. Vasc. Biol. 1999;19:972–978.

[41] Rienstra M, Sun JX, Magnani JW, et al. White blood cell count and risk of incident atrial fibrillation (from the Framingham Heart Study). Am. J. Cardiol. 2012;109:533–537.

[42] Korantzopoulos P, Kolettis TM, Kountouris E, et al. Variation of inflammatory indexes after electrical cardioversion of persistent atrial fibrillation. Is there an association with early recurrence rates? Int. J. Clin. Pract. 2005;59:881–885.

[43] Weymann A, Ali-Hasan-Al-Saegh S, Sabashnikov A, et al. Prediction of New-Onset and Recurrent Atrial Fibrillation by Complete Blood Count Tests: A Comprehensive Systematic Review with Meta-Analysis. Med. Sci. Monit. Basic Res. 2017;23:179–222.

[44] Suzuki A, Fukuzawa K, Yamashita T, et al. Circulating intermediate CD14++CD16+ monocytes are increased in patients with atrial fibrillation and reflect the functional remodelling of the left atrium. Europace. 2017;19:40–47.

[45] Nozawa T, Inoue H, Hirai T, et al. D-dimer level influences thromboembolic events in patients with atrial fibrillation. Int. J. Cardiol. 2006;109:59–65.

[46] Vene N, Mavri A, Košmelj K, et al. High D-dimer levels predict cardiovascular events in patients with chronic atrial fibrillation during oral anticoagulant therapy. Thromb Haemost. 2003;90:1163–1172.

[47] Sadanaga T, Sadanaga M, Ogawa S. Evidence That D-Dimer Levels Predict Subsequent Thromboembolic and Cardiovascular Events in Patients With Atrial Fibrillation During Oral Anticoagulant Therapy. J. Am. Coll. Cardiol. 2010;55:2225–2231.

[48] You L-R, Tang M. The association of high D-dimer level with high risk of ischemic stroke in nonvalvular atrial fibrillation patients: A retrospective study. Medicine (Baltimore). 2018;97.

[49] Rivera-Caravaca JM, Roldán V, Romera M, et al. Soluble Fibrin Monomer Complex and Prediction of Cardiovascular Events in Atrial Fibrillation: The Observational Murcia Atrial Fibrillation Project. J. Gen. Intern. Med. 2018;33:847–854.

[50] Refaai MA, Riley P, Mardovina T, et al. The Clinical Significance of Fibrin Monomers. Thromb. Haemost. 2018;118:1856–1866.

[51] Ancedy Y, Berthelot E, Lang S, et al. Is von Willebrand factor associated with stroke and death at mid-term in patients with non-valvular atrial fibrillation? Arch. Cardiovasc. Dis. 2018;111:357–369.

[52] García-Fernández A, Roldán V, Rivera-Caravaca JM, et al. Does von Willebrand factor improve the predictive ability of current risk stratification scores in patients with atrial fibrillation? Sci. Rep. 2017;7:41565.

[53] Rivera-Caravaca JM, Marín F, Vilchez JA, et al. Refining Stroke and Bleeding Prediction in Atrial Fibrillation by Adding Consecutive Biomarkers to Clinical Risk Scores. Stroke. 2019;50:1372–1379.

\* Study trying to further refine CHA2DS2-VASc score using blood based biomarkers.

[54] Conway DSG, Heeringa J, Van Der Kuip DAM, et al. Atrial fibrillation and the prothrombotic state in the elderly: The Rotterdam study. Stroke. 2003;34:413–417.

[55] Heppell RM, Berkin KE, McLenachan JM, et al. Haemostatic and haemodynamic abnormalities associated with left atrial thrombosis in non-rheumatic atrial fibrillation. Heart. 1997;77:407 LP – 411.

[56] Yaghi S, Song C, Gray WA, et al. Left Atrial Appendage Function and Stroke Risk. Stroke. 2015/10/27. 2015;46:3554–3559.

[57] Ehrlich JR, Kaluzny M, Baumann S, et al. Biomarkers of structural remodelling and endothelial dysfunction for prediction of cardiovascular events or death in patients with atrial fibrillation. Clin. Res. Cardiol. 2011;100:1029–1036.

[58] Kodani E, Inoue H, Atarashi H, et al. Impact of hemoglobin concentration and platelet count on outcomes of patients with non-valvular atrial fibrillation: A subanalysis of the J-RHYTHM Registry. Int. J. Cardiol. 2020;302:81–87.

[59] Park J, Cha M-J, Choi Y, et al. Prognostic efficacy of platelet count in patients with nonvalvular atrial fibrillation. Hear. Rhythm. 2019;16:197–203.

[60] Choudhury A, Chung I, Blann AD, et al. Elevated platelet microparticle levels in nonvalvular atrial fibrillation: Relationship to P-selectin and antithrombotic therapy. Chest. 2007;131:809–815.

[61] Kamath S, Blann AD, Chin BSP, et al. Platelet activation, haemorheology and thrombogenesis in acute atrial fibrillation: a comparison with permanent atrial fibrillation. Heart. 2003;89:1093–1095.

[62] Kaplan KL, Nossel HL, Drillings M, et al. Radioimmunoassay of Platelet Factor 4 and β‐Thromboglobulin: Development and Application to Studies of Platelet Release in Relation to Fibrinopeptide A Generation. Br. J. Haematol. 1978;39:129–146.

[63] Minamino T, Kitakaze M, Asanuma H, et al. Plasma adenosine levels and platelet activation in patients with atrial fibrillation. Am. J. Cardiol. 1999;83:194–198.

[64] Feinberg WM, Pearce LA, Hart RG, et al. Markers of thrombin and platelet activity in patients with atrial fibrillation: Correlation with stroke among 1531 participants in the stroke prevention in atrial fibrillation III study. Stroke. 1999;30:2547–2553.

[65] Agrotis A, Kalinina N, Bobik A. Transforming Growth Factor-β, Cell Signaling and Cardiovascular Disorders. Curr. Vasc. Pharmacol. 2005;3:55–61.

[66] Hayashi H, Sakai T. Biological significance of local TGF-β activation in liver diseases. Front. Physiol. 2012. p. 12.

[67] Aihara KI, Ikeda Y, Yagi S, et al. Transforming growth factor-β1 as a common target molecule for development of cardiovascular diseases, renal insufficiency and metabolic syndrome. Cardiol. Res. Pract. 2011. p. 175381.

[68] Biernacka A, Dobaczewski M, Frangogiannis NG. TGF-β signaling in fibrosis. Growth Factors. 2011/07/11. 2011;29:196–202.

[69] Evans RA, Tian Y. C, Steadman R, et al. TGF-β1-mediated fibroblast–myofibroblast terminal differentiation—the role of smad proteins. Exp. Cell Res. 2003;282:90–100.

[70] Ramos-Mondragón R, Galindo CA, Avila G. Role of TGF-beta on cardiac structural and electrical remodeling. Vasc. Health Risk Manag. 2008;4:1289–1300.

[71] Li J, Yang Y, Ng CY, et al. Association of Plasma Transforming Growth Factor-β1 Levels and the Risk of Atrial Fibrillation: A Meta-Analysis. PLoS One. 2016;11:e0155275.

[72] Lin X, Wu N, Shi Y, et al. Association between transforming growth factor β1 and atrial fibrillation in essential hypertensive patients. Clin. Exp. Hypertens. 2015;37:82–87.

[73] Heinzmann D, Fuß S, Ungern-Sternberg S V, et al. TGFβ Is Specifically Upregulated on Circulating CD14 ++ CD16 + and CD14 + CD16 ++ Monocytes in Patients with Atrial Fibrillation and Severe Atrial Fibrosis. Cell. Physiol. Biochem. 2018;49:226–234.

[74] Tian Y, Wang Y, Chen W, et al. Role of serum TGF-β1 level in atrial fibrosis and outcome after catheter ablation for paroxysmal atrial fibrillation. Medicine (Baltimore). 2017;96.

[75] Artz A, Butz S, Vestweber D. GDF-15 inhibits integrin activation and mouse neutrophil recruitment through the ALK-5/TGF-βRII heterodimer. Blood. 2016;128:529–541.

[76] Yang H, Filipovic Z, Brown D, et al. Macrophage inhibitory cytokine-1: A novel biomarker for p53 pathway activation. Mol. Cancer Ther. 2003;2:1023 LP – 1029.

[77] Sharma A, Hijazi Z, Andersson U, et al. Use of biomarkers to predict specific causes of death in patients with Atrial fibrillation: Insights from the Aristotle Trial. Circulation. 2018;138:1666–1676.

[78] Hijazi Z, Oldgren J, Andersson U, et al. Growth-differentiation factor 15 and risk of major bleeding in atrial fibrillation: Insights from the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial. Am. Heart J. 2017;190:94–103.

[79] Wallentin L, Hijazi Z, Andersson U, et al. Growth differentiation factor 15, a marker of oxidative stress and inflammation, for risk assessment in patients with atrial fibrillation: Insights from the Apixaban for reduction in stroke and other thromboembolic events in atrial fibrillation (ARISTOTLE. Circulation. 2014;130:1847–1858.

[80] Hu XF, Zhan R, Xu S, et al. Growth differentiation factor 15 is associated with left atrial/left atrial appendage thrombus in patients with nonvalvular atrial fibrillation. Clin. Cardiol. 2018;41:34–38.

[81] Hijazi Z, Oldgren J, Andersson U, et al. Growth-differentiation factor 15 and risk of major bleeding in atrial fibrillation: Insights from the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial. Am. Heart J. 2017;190:94–103.

[82] Wallentin L, Hijazi Z, Andersson U, et al. Growth differentiation factor 15, a marker of oxidative stress and inflammation, for risk assessment in patients with atrial fibrillation: Insights from the Apixaban for reduction in stroke and other thromboembolic events in atrial fibrillation (ARISTOTLE. Circulation. 2014;130:1847–1858.

[83] Li L, Li J, Gao J. Functions of Galectin-3 and Its Role in Fibrotic Diseases. J. Pharmacol. Exp. Ther. 2014;351:336 LP – 343.

[84] Lippi G, Cervellin G, Sanchis-Gomar F. Galectin-3 in atrial fibrillation: Simple bystander, player or both? Clin. Biochem. 2015;48:818–822.

[85] Hernández-Romero D, Vílchez JA, Lahoz Á, et al. Galectin-3 as a marker of interstitial atrial remodelling involved in atrial fibrillation. Sci. Rep. 2017;7.

[86] Takemoto Y, Ramirez RJ, Yokokawa M, et al. Galectin-3 Regulates Atrial Fibrillation Remodeling and Predicts Catheter Ablation Outcomes. JACC. Basic to Transl. Sci. 2016;1:143–154.

[87] Gong M, Cheung A, Wang Q-S, et al. Galectin-3 and risk of atrial fibrillation: A systematic review and meta-analysis. J. Clin. Lab. Anal. 2020;n/a:e23104.

[88] Geenen LW, Baggen VJM, van den Bosch AE, et al. Prognostic value of soluble ST2 in adults with congenital heart disease. Heart. 2019;105:999 LP – 1006.

[89] Weinberg EO, Shimpo M, Hurwitz S, et al. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. Circulation. 2003;107:721–726.

[90] AlTurki A. Soluble ST2 in Paroxysmal Atrial Fibrillation: a New Biomarker that Predicts Recurrence? Korean Circ. J. 2018;48:930–932.

[91] Rienstra M, Yin X, Larson MG, et al. Relation between soluble ST2, growth differentiation factor–15, and high-sensitivity troponin I and incident atrial fibrillation. Am. Heart J. 2014;167:109-115.e2.

[92] Vílchez JA, Pérez-Cuellar M, Marín F, et al. sST2 levels are associated with all-cause mortality in anticoagulated patients with atrial fibrillation. Eur. J. Clin. Invest. 2015;45:899–905.

[93] Okar S, Kaypakli O, Sahin DY, et al. Fibrosis marker soluble ST2 predicts atrial fibrillation recurrence after cryoballoon catheter ablation of nonvalvular paroxysmal atrial fibrillation. Korean Circ. J. 2018;48:920–929.

[94] Chen C, Qu X, Gao Z, et al. Soluble ST2 in patients with nonvalvular atrial fibrillation and prediction of heart failure. Int. Heart J. 2018;59:58–63.

[95] Klein T, Bischoff R. Physiology and pathophysiology of matrix metalloproteases. Amino Acids. 2011;41:271–290.

[96] Li M, Yang G, Xie B, et al. Changes in matrix metalloproteinase-9 levels during progression of atrial fibrillation. J. Int. Med. Res. 2013;42:224–230.

[97] Nakano Y, Niida S, Dote K, et al. Matrix metalloproteinase-9 contributes to human atrial remodeling during atrial fibrillation. J. Am. Coll. Cardiol. 2004;43:818–825.

[98] Saura D, Marín F, Climent V, et al. Left atrial remodelling in hypertrophic cardiomyopathy: relation with exercise capacity and biochemical markers of tissue strain and remodelling. Int. J. Clin. Pract. 2009;63:1465–1471.

[99] Sasaki N, Okumura Y, Watanabe I, et al. Increased levels of inflammatory and extracellular matrix turnover biomarkers persist despite reverse atrial structural remodeling during the first year after atrial fibrillation ablation. J. Interv. Card. Electrophysiol. 2014;39:241–249.

[100] Marín F, Pascual DA, Roldán V, et al. Statins and Postoperative Risk of Atrial Fibrillation Following Coronary Artery Bypass Grafting. Am. J. Cardiol. 2006;97:55–60.

[101] Marín F, Roldán V, Climent V, et al. Is thrombogenesis in atrial fibrillation related to matrix metalloproteinase-1 and its inhibitor, TIMP-1? Stroke. 2003;34:1181–1186.

[102] Anné W, Willems R, Roskams T, et al. Matrix metalloproteinases and atrial remodeling in patients with mitral valve disease and atrial fibrillation. Cardiovasc. Res. 2005;67:655–666.

[103] Lip GYH, Nieuwlaat R, Pisters R, et al. Refining clinical risk stratification for predicting stroke and thromboembolism in atrial fibrillation using a novel risk factor-based approach: The Euro Heart Survey on atrial fibrillation. Chest. 2010;137:263–272.

\*\* Development of the CHA2DS2-VASc score

[104] Borre ED, Goode A, Raitz G, et al. Predicting Thromboembolic and Bleeding Event Risk in Patients with Non-Valvular Atrial Fibrillation: A Systematic Review. Thromb. Haemost. 2018;118:2171–2187.

[105] Lip GYH, Lane D, Van Walraven C, et al. Additive role of plasma von Willebrand factor levels to clinical factors for risk stratification of patients with atrial fibrillation. Stroke. 2006;37:2294–2300.

[106] Roldán V, Rivera-Caravaca JM, Shantsila A, et al. Enhancing the ‘real world’ prediction of cardiovascular events and major bleeding with the CHA 2 DS 2 -VASc and HAS-BLED scores using multiple biomarkers. Ann. Med. 2018;50:26–34.

[107] Hijazi Z, Lindbäck J, Alexander JH, et al. The ABC (age, biomarkers, clinical history) stroke risk score: a biomarker-based risk score for predicting stroke in atrial fibrillation. Eur. Heart J. 2016;37:1582–1590.

\*\* ABC score for the assessment of stroke risk.

[108] Jonas O, Ziad H, Johan L, et al. Performance and Validation of a Novel Biomarker-Based Stroke Risk Score for Atrial Fibrillation. Circulation. 2016;134:1697–1707.

[109] Berg DD, Ruff CT, Jarolim P, et al. Performance of the ABC Scores for Assessing the Risk of Stroke or Systemic Embolism and Bleeding in Patients With Atrial Fibrillation in ENGAGE AF-TIMI 48. Circulation. 2019;139:760–771.

[110] Rivera-Caravaca JM, Roldán V, Esteve-Pastor MA, et al. Long-term stroke risk prediction in patients with atrial fibrillation: Comparison of the ABC-stroke and CHA2DS2-VASc scores. J. Am. Heart Assoc. 2017;6:e006490.

[111] Pisters R, Lane DA, Nieuwlaat R, et al. A Novel User-Friendly Score (HAS-BLED) To Assess 1-Year Risk of Major Bleeding in Patients With Atrial Fibrillation: The Euro Heart Survey. Chest. 2010;138:1093–1100.

[112] Hijazi Z, Oldgren J, Lindbäck J, et al. The novel biomarker-based ABC (age, biomarkers, clinical history)-bleeding risk score for patients with atrial fibrillation: a derivation and validation study. Lancet. 2016;387:2302–2311.

[113] Esteve-Pastor MA, Rivera-Caravaca JM, Roldán V, et al. Long-term bleeding risk prediction in ‘real world’ patients with atrial fibrillation: Comparison of the HAS-BLED and abc-bleeding risk scores: The murcia atrial fibrillation project. Thromb. Haemost. 2017;117:1848–1858.

[114] Hijazi Z, Oldgren J, Lindbäck J, et al. A biomarker-based risk score to predict death in patients with atrial fibrillation: The ABC (age, biomarkers, clinical history) death risk score. Eur. Heart J. 2018;39:477–485.

[115] Camelo-Castillo A, Rivera-Caravaca J, Marín F, et al. Predicting adverse events beyond stroke and bleeding with the ABC-stroke and ABC-bleeding scores in patients with atrial fibrillation: The Murcia AF Project. Thromb. Haemost. 2020;

[116] Rivera-Caravaca JM, Esteve-Pastor MA, Marín F, et al. A Propensity Score Matched Comparison of Clinical Outcomes in Atrial Fibrillation Patients Taking Vitamin K Antagonists: Comparing the “Real-World” vs Clinical Trials. Mayo Clin. Proc. 2018;93:1065–1073.

[117] Chao TF, Liao JN, Tuan TC, et al. Incident Co-Morbidities in Patients with Atrial Fibrillation Initially with a CHA 2 DS 2 -VASc Score of 0 (Males) or 1 (Females): Implications for Reassessment of Stroke Risk in Initially “Low-Risk” Patients. Thromb. Haemost. 2019;119:1162–1170.

[118] Yoon M, Yang PS, Jang E, et al. Dynamic Changes of CHA 2 DS 2 -VASc Score and the Risk of Ischaemic Stroke in Asian Patients with Atrial Fibrillation: A Nationwide Cohort Study. Thromb. Haemost. 2018;118:1296–1304.

[119] Chao T-F, Lip GYH, Lin Y-J, et al. Incident Risk Factors and Major Bleeding in Patients with Atrial Fibrillation Treated with Oral Anticoagulants: A Comparison of Baseline, Follow-up and Delta HAS-BLED Scores with an Approach Focused on Modifiable Bleeding Risk Factors. Thromb. Haemost. 2018;118:768–777.

[120] Esteve-Pastor MA, Roldán V, Rivera-Caravaca JM, et al. The Use of Biomarkers in Clinical Management Guidelines: A Critical Appraisal. Thromb. Haemost. 2019;119:1901–1919.

\*\* Paper reviews and evaluates the use of biomarkers in clinical management in cardiovascular diseases.

[121] Rivera-Caravaca JM, Esteve-Pastor MA. Heart Failure and Cardiac Events: Is a Consecutive Measurement of Biomarkers a Simple and Practical Approach? Thromb. Haemost. 2019;119:1891–1893.

[122] Gupta DK, Shah AM, Giugliano RP, et al. Left atrial structure and function in atrial fibrillation: ENGAGE AF-TIMI 48. Eur. Heart J. 2013;35:1457–1465.

[123] Lim DJ, Ambale-Ventakesh B, Ostovaneh MR, et al. Change in left atrial function predicts incident atrial fibrillation: the Multi-Ethnic Study of Atherosclerosis. Eur. Hear. J. - Cardiovasc. Imaging. 2019;20:979–987.

[124] Hamatani Y, Ogawa H, Takabayashi K, et al. Left atrial enlargement is an independent predictor of stroke and systemic embolism in patients with non-valvular atrial fibrillation. Sci. Rep. 2016;6:31042.

[125] Dakay K, Chang AD, Hemendinger M, et al. Left Atrial Enlargement and Anticoagulation Status in Patients with Acute Ischemic Stroke and Atrial Fibrillation. J. Stroke Cerebrovasc. Dis. 2018;27:192–197.

[126] Jin X, Pan J, Wu H, et al. Are left ventricular ejection fraction and left atrial diameter related to atrial fibrillation recurrence after catheter ablation?: A meta-analysis. Medicine (Baltimore). 2018;97.

[127] Oka T, Tanaka K, Ninomiya Y, et al. Impact of baseline left atrial function on long-term outcome after catheter ablation for paroxysmal atrial fibrillation. J. Cardiol. 2020;75:352–359.

[128] Shih J-Y, Tsai W-C, Huang Y-Y, et al. Association of Decreased Left Atrial Strain and Strain Rate with Stroke in Chronic Atrial Fibrillation. J. Am. Soc. Echocardiogr. 2011;24:513–519.

[129] Cameli M, Lunghetti S, Mandoli GE, et al. Left atrial strain predicts pro-thrombotic state in patients with non-valvular atrial fibrillation. J. Atr. Fibrillation. 2017;10.

[130] Saha SK, Anderson PL, Caracciolo G, et al. Global Left Atrial Strain Correlates with CHADS2 Risk Score in Patients with Atrial Fibrillation. J. Am. Soc. Echocardiogr. 2011;24:506–512.

[131] Rasmussen SMA, Olsen FJ, Jørgensen PG, et al. Utility of left atrial strain for predicting atrial fibrillation following ischemic stroke. Int. J. Cardiovasc. Imaging. 2019;35:1605–1613.

[132] Skaarup KG, Christensen H, Høst N, et al. Usefulness of left ventricular speckle tracking echocardiography and novel measures of left atrial structure and function in diagnosing paroxysmal atrial fibrillation in ischemic stroke and transient ischemic attack patients. Int. J. Cardiovasc. Imaging. 2017;33:1921–1929.

[133] Blackshear JL, Odell JA. Appendage obliteration to reduce stroke in cardiac surgical patients with atrial fibrillation. Ann. Thorac. Surg. 1996;61:755–759.

[134] Stoddard MF, Dawkins PR, Prince CR, et al. Left atrial appendage thrombus is not uncommon in patients with acute atrial fibrillation and a recent embolic event: A transesophageal echocardiographics tudy. J. Am. Coll. Cardiol. 1995;25:452–459.

[135] Di Biase L, Santangeli P, Anselmino M, et al. Does the Left Atrial Appendage Morphology Correlate With the Risk of Stroke in Patients With Atrial Fibrillation?: Results From a Multicenter Study. J. Am. Coll. Cardiol. 2012;60:531–538.

[136] Kong B, Liu Y, Hu H, et al. Left atrial appendage morphology in patients with atrial fibrillation in China: implications for stroke risk assessment from a single center study. Chin. Med. J. (Engl). 2014;127.

[137] Kimura T, Takatsuki S, Inagawa K, et al. Anatomical characteristics of the left atrial appendage in cardiogenic stroke with low CHADS2 scores. Hear. Rhythm. 2013;10:921–925.

[138] Khurram IM, Dewire J, Mager M, et al. Relationship between left atrial appendage morphology and stroke in patients with atrial fibrillation. Hear. Rhythm. 2013;10:1843–1849.

[139] Vergara GR, Marrouche NF. Tailored management of atrial fibrillation using a LGE-MRI based model: From the clinic to the electrophysiology laboratory. J. Cardiovasc. Electrophysiol. 2011;22:481–487.

[140] Benito EM, Cabanelas N, Nuñez-Garcia M, et al. Preferential regional distribution of atrial fibrosis in posterior wall around left inferior pulmonary vein as identified by late gadolinium enhancement cardiac magnetic resonance in patients with atrial fibrillation. Europace. 2018;20:1959–1965.

[141] Lee DK, Shim J, Choi J-I, et al. Left atrial fibrosis assessed with cardiac mri in patients with paroxysmal and those with persistent atrial fibrillation. Radiology. 2019;292:575–582.

[142] Akoum N, Fernandez G, Wilson B, et al. Association of atrial fibrosis quantified using LGE-MRI with atrial appendage thrombus and spontaneous contrast on transesophageal echocardiography in patients with atrial fibrillation. J. Cardiovasc. Electrophysiol. 2013/07/11. 2013;24:1104–1109.

[143] McGann C, Akoum N, Patel A, et al. Atrial fibrillation ablation outcome is predicted by left atrial remodeling on MRI. Circ. Arrhythmia Electrophysiol. 2014;7:23–30.

[144] Csécs I, Yamaguchi T, Kheirkhahan M, et al. Left atrial functional and structural changes associated with ablation of atrial fibrillation - Cardiac magnetic resonance study. Int. J. Cardiol. 2020;305:154–160.

[145] Khurram IM, Habibi M, Gucuk Ipek E, et al. Left Atrial LGE and Arrhythmia Recurrence Following Pulmonary Vein Isolation for Paroxysmal and Persistent AF. JACC Cardiovasc. Imaging. 2016;9:142–148.

[146] Sramko M, Peichl P, Wichterle D, et al. Clinical value of assessment of left atrial late gadolinium enhancement in patients undergoing ablation of atrial fibrillation. Int. J. Cardiol. 2015;179:351–357.

[147] Roselli C, Michiel R, Ellinor PT. Genetics of Atrial Fibrillation in 2020. Circ. Res. 2020;127:21–33.

\*\* Provides comprehensive overview of the involvement of genetics in AF.

[148] Feghaly J, Zakka P, London B, et al. Genetics of Atrial Fibrillation. J. Am. Heart Assoc. 2018;7:e009884.

\*\* Provides comprehensive overview of the involvement of genetics in AF.

[149] Shoemaker MB, Shah RL, Roden DM, et al. How Will Genetics Inform the Clinical Care of Atrial Fibrillation? Circ. Res. 2020;127:111–127.

\*\*Review article on how genetics can inform clinical care in AF.

[150] Fox CS, Parise H, D’Agostino Ralph B. S, et al. Parental Atrial Fibrillation as a Risk Factor for Atrial Fibrillation in Offspring. JAMA. 2004;291:2851–2855.

[151] Arnar DO, Thorvaldsson S, Manolio TA, et al. Familial aggregation of atrial fibrillation in Iceland. Eur. Heart J. 2006;27:708–712.

\* Study identifying relative risk of developing AF with first degree family members

[152] Øyen N, Ranthe MF, Carstensen L, et al. Familial Aggregation of Lone Atrial Fibrillation in Young Persons. J. Am. Coll. Cardiol. 2012;60:917–921.

[153] Yang Y, Xia M, Jin Q, et al. Identification of a KCNE2 gain-of-function mutation in patients with familial atrial fibrillation. Am. J. Hum. Genet. 2004/09/13. 2004;75:899–905.

[154] Xia M, Jin Q, Bendahhou S, et al. A Kir2.1 gain-of-function mutation underlies familial atrial fibrillation. Biochem. Biophys. Res. Commun. 2005;332:1012–1019.

[155] Olson TM, Alekseev AE, Liu XK, et al. Kv1.5 channelopathy due to KCNA5 loss-of-function mutation causes human atrial fibrillation . Hum. Mol. Genet. 2006;15:2185–2191.

[156] Otway R, Vandenberg JI, Guo G, et al. Stretch-Sensitive KCNQ1Mutation: A Link Between Genetic and Environmental Factors in the Pathogenesis of Atrial Fibrillation? J. Am. Coll. Cardiol. 2007;49:578–586.

[157] Chen Y-H, Xu S-J, Bendahhou S, et al. KCNQ1 Gain-of-Function Mutation in Familial Atrial Fibrillation. Science (80-. ). 2003;299:251 LP – 254.

[158] Dawood D, J. KP, S. DB, et al. Cardiac Sodium Channel (SCN5A) Variants Associated with Atrial Fibrillation. Circulation. 2008;117:1927–1935.

[159] Chen Y-H, Xu S-J, Bendahhou S, et al. KCNQ1 Gain-of-Function Mutation in Familial Atrial Fibrillation. Science (80-. ). 2003;299:251 LP – 254.

[160] Lubitz SA, Yi BA, Ellinor PT. Genetics of atrial fibrillation. Heart Fail. Clin. 2010;6:239–247.

[161] Ellinor PT, Petrov-Kondratov VI, Zakharova E, et al. Potassium channel gene mutations rarely cause atrial fibrillation. BMC Med. Genet. 2006;7:70.

[162] Gudbjartsson DF, Arnar DO, Helgadottir A, et al. Variants conferring risk of atrial fibrillation on chromosome 4q25. Nature. 2007;448:353.

[163] Paludan-Müller C, Svendsen JH, Olesen MS. The role of common genetic variants in atrial fibrillation. J. Electrocardiol. 2016;49:864–870.

[164] Kääb S, Darbar D, van Noord C, et al. Large scale replication and meta-analysis of variants on chromosome 4q25 associated with atrial fibrillation. Eur. Heart J. 2009;30:813–819.

[165] Gudbjartsson DF, Holm H, Gretarsdottir S, et al. A sequence variant in ZFHX3 on 16q22 associates with atrial fibrillation and ischemic stroke. Nat. Genet. 2009/07/13. 2009;41:876–878.

[166] Benjamin EJ, Rice KM, Arking DE, et al. Variants in ZFHX3 are associated with atrial fibrillation in individuals of European ancestry. Nat. Genet. 2009;41:879–881.

[167] Huang Y, Wang C, Yao Y, et al. Molecular Basis of Gene-Gene Interaction: Cyclic Cross-Regulation of Gene Expression and Post-GWAS Gene-Gene Interaction Involved in Atrial Fibrillation. PLOS Genet. 2015;11:e1005393.

[168] Gretarsdottir S, Thorleifsson G, Manolescu A, et al. Risk variants for atrial fibrillation on chromosome 4q25 associate with ischemic stroke. Ann. Neurol. 2008;64:402–409.

[169] Lemmens R, Buysschaert I, Geelen V, et al. The association of the 4q25 susceptibility variant for atrial fibrillation with stroke is limited to stroke of cardioembolic etiology. Stroke. 2010;41:1850–1857.

[170] Marín F, Corral J, Roldán V, et al. Factor XIII Val34Leu polymorphism modulates the prothrombotic and inflammatory state associated with atrial fibrillation. J. Mol. Cell. Cardiol. 2004;37:699–704.

[171] Hu X, Wang J, Li Y, et al. The β-fibrinogen gene 455G/A polymorphism associated with cardioembolic stroke in atrial fibrillation with low CHA2DS2-VaSc score. Sci. Rep. 2017;7:17517.

[172] Roldán V, Marín F, González-Conejero R, et al. Factor VII –323 decanucleotide D/I polymorphism in atrial fibrillation: Implications for the prothrombotic state and stroke risk. Ann. Med. 2008;40:553–559.

[173] Go AS, Reed GL, Hylek EM, et al. Factor V Leiden and Risk of Ischemic Stroke in Nonvalvular Atrial Fibrillation: The AnTicoagulation and Risk Factors in Atrial Fibrillation (ATRIA) Study. J. Thromb. Thrombolysis. 2003;15:41–46.

[174] Roldán V, Arroyo AB, Salloum-Asfar S, et al. Prognostic role of MIR146A polymorphisms for cardiovascular events in atrial fibrillation. Thromb. Haemost. 2014;112:781–788.

[175] Arroyo AB, De Los Reyes-García AM, Rivera-Caravaca JM, et al. MiR-146a Regulates Neutrophil Extracellular Trap Formation That Predicts Adverse Cardiovascular Events in Patients with Atrial Fibrillation. Arterioscler. Thromb. Vasc. Biol. 2018;38:892–902.

[176] Yoon M, Yang P-S, Jang E, et al. Improved Population-Based Clinical Outcomes of Patients with Atrial Fibrillation by Compliance with the Simple ABC (Atrial Fibrillation Better Care) Pathway for Integrated Care Management: A Nationwide Cohort Study. Thromb. Haemost. 2019;19:1695–1703.

[177] Proietti M, Mujovic N, Potpara TS. Optimizing Stroke and Bleeding Risk Assessment in Patients with Atrial Fibrillation: A Balance of Evidence, Practicality and Precision. Thromb. Haemost. 2018;118:2014–2017.

[178] Hylek EM. Treatment Persistence in Atrial Fibrillation: The Next Major Hurdle. Thromb. Haemost. 2018;118:2018–2019.

[179] Sharma A, Hijazi Z, Andersson U, et al. Use of biomarkers to predict specific causes of death in patients with Atrial fibrillation: Insights from the Aristotle Trial. Circulation. 2018;138:1666–1676.

[180] Leung M, van Rosendael PJ, Abou R, et al. Left atrial function to identify patients with atrial fibrillation at high risk of stroke: new insights from a large registry. Eur. Heart J. 2017;39:1416–1425.

[181] Chelu MG, King JB, Kholmovski EG, et al. Atrial fibrosis by late gadolinium enhancement magnetic resonance imaging and catheter ablation of atrial fibrillation: 5-year follow-up data. J. Am. Heart Assoc. 2018;7:e006313.

[182] Suksaranjit P, Marrouche NF, Han FT, et al. Relation of Left Atrial Appendage Remodeling by Magnetic Resonance Imaging and Outcome of Ablation for Atrial Fibrillation. Am. J. Cardiol. 2018;122:83–88.

**Table 1. Blood-based biomarkers in atrial fibrillation**.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Author, year | Population | n | Study outcomes | Follow-up | Biomarkers | Findings |
| Conway, 2004 [31] | AF attending clinic | 77 | Stroke and death | 6.3 years | IL-6  CRP | Increased IL-6 levels were associated with a 2.9-fold increase in risk of stroke or death.  No association between CRP and endpoints. |
| Nozawa, 2006 [45] | AF | 509 | Composite of clinically evident stroke, TIA and SE | 2 years | D-dimer  F1+2  Platelet factor 4  Β-thrombomodulin | Higher D-dimer levels associated with greater risk of endpoint.  No association with endpoint.  No association with endpoint.  No association with endpoint |
| Sadanaga, 2010 [47] | AF on warfarin | 269 | TE events and CV events | 756 days | D-dimer | High D-dimer levels conferred a 15.8-fold increased risk of a TE event and a 7.6-fold increase in risk of a CV event. |
| Van den Bos, 2011 [22] | AF (STEMI excluded) | 407 | All-cause mortality, death, MI, MACE | 688 days | cTnI | Multivariate analysis minor (0.15-0.65ng/ml) and positive (≥0.65ng/ml) elevations in cTnI independently associated with death (HR 2.36).  Independent correlation between combined endpoints of death/MI and MACE for both minor and positive cTnI. |
| Ehrlich, 2011 [57] | AF | 278 | Composite of cardiovascular events (MI, stroke, PE, death) | 28 months | hsCRP  sVCAM-1  MMP-2  sCD40L  vWF | No association of hsCRP with composite endpoint  Higher sVCAM-1 independently associated with composite endpoint.  Higher MMP-2 independently associated with composite endpoint.  No association of sCD40L with composite endpoint.  No association of vWF with composite endpoint. |
| Hijazi, 2012 [20]  RE-LY trial | AF with at least 1 risk factor for stroke | 6 189 | Stroke or SE,  mortality, MI, composite TE end point. | 2.2 years | cTnI  NT-proBNP | Higher troponin I associated with greater risk of stroke, mortality, MI and composite TE endpoint.  Highest quartile associated with 2-fold risk of stroke, 4.4-fold risk of vascular mortality, 3-fold risk of MI and 3.4-fold risk of TE compared to lowest quartile.  Higher NT-proBNP associated with greater risk of stroke, mortality and the composite TE endpoint.  Highest quartile associated with 2.4-fold risk of stroke,6.7-fold risk of vascular death and 3.6-fold risk of TE compared to the lowest quartile. |
| Roldan, 2012 [21] | AF on OAC | 930 | Adverse cardiovascular events (stroke/TIA, SE, ACS, AHF, cardiac death) |  | hsTnT  IL-6 | Higher hsTnT associated with greater risk of an adverse cardiac event or mortality.  Higher IL-6 associated with greater risk of an adverse cardiac event or mortality.  Addition of hsTnT and/or IL-6 to CHA2DS2-VASc improved predictive performance. |
| Hijazi, 2013 [24]  ARISTOTLE trial | AF with at least 1 CHADS2 risk factor | 14892 | Stroke and SE,  all-cause mortality,  cardiac mortality,  major bleeding | 1.9 years | NT-proBNP | Higher NT-proBNP led to increased risk between lowest and highest quartile in all outcome measures apart from major bleeding. Stroke and SE = 2.4-fold  All-cause mortality = 2.25-fold  Cardiac mortality = 2.5-fold  Adding NT-proBNP to CHA2DS2-VASc improves predictive value. |
| Wallentin, 2014 [79]  ARISTOTLE trial | AF with at least 1 risk factor for stroke | 14798 | Stroke, mortality, major bleeding | 1.9 years | GDF-15 | Highest quartile of GDF-15 associated with stroke, mortality and major bleeding compared to lowest quartile. Once adjusted for other biomarkers remained independently associated with major bleeding and mortality. |
| Rienstra, 2014 [91]  Framingham Heart Study | No known heart disease | 3217 | Development of AF | 10 years | sST2  GDF-15  hsTnI | GDF-15 and hsTnI associated with incident AF.  After adjustment for BNP and CRP only hsTnI remained independently associated with AF onset. |
| Roldan, 2014 [25] | AF on OAC | 1172 | Stroke/TIA, major bleeding, all-cause death, composite of cardiovascular endpoints (stroke/TIA, SE, ACS. AHF, cardiac death). | 1007 days | NT-proBNP | Higher NT-proBNP led to increased risk of:  Stroke = 2.7 fold  CV events = 1.9 fold  All cause mortality= 1.7 fold  NT-proBNP was not predictive of bleeding.  NT-proBNP resulted in improved performance for endpoints beyond CHA2DS2-VASc score. |
| Vilchez, 2015 [92] | Permanent AF on OAC | 562 | All-cause mortality and CV events. | 1587 days | sST2 | Independently associated with all-cause mortality. |
| Aulin, 2015 [38]  RE-LY trial | AF with at least 1 stroke risk factor | 6187 | Stroke or SE, MI, vascular death, composite TE outcome | 2 years | IL-6  CRP  Fibrinogen | IL-6 independently associated with stroke or SE, vascular death and composite TE.  Further adjusted for cardiac and renal biomarkers remained significantly related to vascular death and composite TE.  Adding IL-6 to CHA2DS2-VASc increased C index for stroke from 0.62to 0.64.  CRP independently related to MI, vascular death and composite TE outcome.  Once further adjusted for cardiac and renal biomarkers remained significantly related to MI.  No significant association between fibrinogen and outcome measures. |
| Ancedy, 2017 [51] | Hospitalised with AF | 122 | Composite of stroke, all-cause death and heart failure. | 5 years | vWF | Higher vWF levels associated with greater risk of composite endpoint.  Slight but significant improvement in predictive value of CHA2DS2-VASc. |
| Garcia-Fernandez, 2017 [52] | AF on VKA | 1215 | Composite of CV events, stroke/TIA, SE, ACS, acute heart failure and death | 2373 days | vWF | After adjustment vWF significantly associated with incidence of stroke, all-cause mortality and cardiovascular mortality and composite of CV events.  Addition of vWF to CHA2DS2-VASc significantly predictive value for stroke, CV events and cardiovascular mortality but C-indexes not significantly different. |
| Tian, 2017 [74] | Patients undergoing catheter ablation | 98 (38 AF) | AF recurrence | 241 days | TGF-β1  P-III-P  Type 4 pro-collagen  Laminin | Concentrations of TGF-β1, P-III-P, Type 4 procollagen and laminin significantly higher in AF group compared to control group.  TGF-β1 only independent predictor after mulitvariate analysis. |
| Hijazi, 2017 [78]  RE-LY trial | AF with at least 1 risk factor for stroke | 8474 | Stroke, mortality, major bleeding | 1.9 years | GDF-15 | Increased concentrations of GDF-15 associated with stroke, mortality and major bleeding. After adjustment for other biomarkers remained independent predictor for mortality and bleeding. |
| Sharma, 2018 [179]  ARISTOTLE trial | AF with at least 1 risk factor | 14798 | Cause-specific cardiac death.  Death from any cause. | 1.9 years | IL-6  hsTnT  NT-proBNP  GDF-15 | hsTnT and NT-proBNP strongest variables associated with specific cardiac disease.  NT-proBNP and GDF-15 most strongly associated with heart failure death.  Secondary to prior stroke hsTnT was most strongly associated with stroke/SE. |
| Chen, 2018 [94] | NVAF | 174 | Heart failure | 6 months | sST2 | sST2 levels associated with a 5.88-fold risk of heart failure. |
| Rivera-Caravaca, 2019 [53] | AF on VKA | 940 | Ischaemic stroke | 6.5 years | vWF  hscTnT  NT-proBNP  IL6  Fibrin monomers  BTP | VWF, TnT, IL6 and NT-proBNP levels higher in patients with CHA2DS2-VASc ≥2.  Adding markers to CHA2DS2-VASc did not significantly increase predictive performance. |
| Kim, 2020 [23] | New onset AF | 957 | All cause death. Readmission due to heart failure, stroke or revascularization | 19.3 months | cTnI | Increased risk of all-cause death and readmission due to HF and revascularization between 4th and 1st cTnI quartiles but not in stroke readmission.  Independent predictors of all-cause death were renal insufficiency, cTnI in Q4 and anti-coagulant therapy.  Q4 group had higher risk of presenting with persistent AF at last follow-up visit. |

ACS, acute coronary syndrome; AF, atrial fibrillation; AHF, acute heart failure; BTP, β-trace protein; cTnI, cardiac troponin I; CV, cardiovascular; F1+2, prothrombin fragment F1+2; GDF-15, growth differentiation factor-15; hsCRP, high sensitivity C-reactive protein; hsTnI, high sensitivity troponin I;hsTnT, high sensitivity troponin T; IL-6, interleukin 6; MACE, major adverse cardiovascular events; MI, myocardial infarction; MMP2, matrix metallo-proteinase;MMP-2, matrix metalloproteinase-2; NT-proBNP, N-terminal-pro B-type natriuretic peptide; NVAF, non-valvular atrial fibrillation; OAC, oral anti-coagulant; P-III-P, procollagen type 3; PE, pulmonary embolism; sCD40L, soluble CD40 ligand; SE, systemic embolism; sST2, soluble suppression of tumorigenicity 2; STEMI, ST elevation myocardial infarction;sVCAM, soluble vascular cell adhesion molecule; TE, thromboembolism; TGF-β1, transforming growth factor-β 1; TIA, transient ischaemic attack; TIA, transient ischemic attack;VKA, vitamin K antagonist; vWF, von Willebrand factor.

**Table 2. Evidence for imaging biomarkers in atrial fibrillation**.

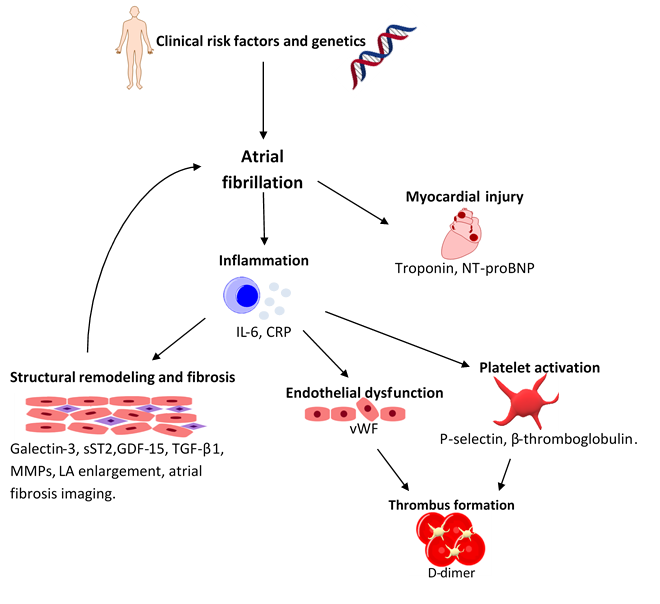
|  |  |  |  |
| --- | --- | --- | --- |
| Author, year | Study population | Imaging technique | Findings |
| Akoum, 2013 [142] | 178 patients with AF prior to ablation or cardioversion | TEE- Thrombi formation  LGE-MRI- atrial fibrosis | Atrial fibrosis independently associated with LAA thrombus. |
| Gupta, 2014 [122] | 971 patients in ENGAGE AF-TIMI 48 sub-study | Echo - LA structure and function | Percentage of population with increased LA size increased with AF burden. LAEF declined across spectrum of AF. LA dysfunction presents even in patients in SR and normal LA volume. |
| Hamatani, 2016 [124] | 2713 patients from Fushimi AF registry | Echo - LA size | Patients with sustained AF had a larger LA diameter than those with paroxysmal AF. LA enlargement independent predictor of stroke/SE. |
| Dakay, 2018 [125] | 225 patients with AF admitted for ischaemic stroke | TTE – LA size | Patients who had a stroke despite therapeutic anti-coagulation more likely to have moderate to severe LA enlargement. |
| Benito, 2018 [140] | 113 patients referred for AF ablation | LGE- MRI – atrial fibrosis | Patients with AF have fibrosis preferentially located at the posterior wall and floor around the antrum of the left inferior pulmonary vein. |
| Leung, 2018 [180] | 1361 patients first diagnosed with AF and followed for 7.9 years. | TTE- LA volumes, LA reservoir strain, PA-TDI | Assessment of LA reservoir strain and PA-TDI provides additional risk stratification for stroke. |
| Chelu, 2018 [181] | 308 patients with AF undergoing ablation. | LGE-MRI – atrial fibrosis | More advanced atrial fibrosis more likely to develop AF recurrence and undergo further ablation. |
| Suksaranjit, 2018 [182] | 74 patients prior to ablation | LGE-MRI – LAA fibrosis | Extent of LAA structural remodelling is associated with arrhythmia recurrence after ablation. |
| Lim, 2019 [123] | 2338 patients free of cardiovascular disease at baseline. | CMRI – LA volume and function over 10 years | Annual decrease of LAEF most strongly associated with risk of AF. |
| Lee, 2019 [141] | 195 patients with paroxysmal or permanent AF. | LGE-MRI – atrial fibrosis | Presence of left atrial pulmonary vein antrum associated with persistent AF. |
| Oka, 2020 [127] | 292 undergoing catheter ablations for paroxysmal AF | CT – LAV and LAEF | LAEF associated with recurrence after ablation for paroxysmal AF. |
| Csecs, 2020 [144] | 55 AF patients pre and 24hr and 3 months post ablation. | CMRI – LA volume and function and LA fibrosis used LGE-MRI imaging | Pre-ablation function inversely correlated to LALGE-MRI imaging and was related to success of ablation. |

AF, atrial fibrillation; CMRI, cardiac magnetic resonance imaging; CT, computed tomography; LA, left atrium; LAEF, left atrial ejection fraction; LAV, left atrial volume; LGE-MRI, late gadolinium enhancement – magnetic resonance imaging; PA-TDI, P to A’ wave – tissue doppler imaging (total atrial conduction time);TEE, transesophageal echocardiogram; LAA, left atrial appendage; TTE, transthoracic echocardiogram; echo, echocardiogram.

**Table 3: Summary of biomarkers associated with atrial fibrillation and outcome measures.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Biomarkers | AF incidence | Stroke risk | Bleeding risk | All-cause mortality | Recurrence |
| NT-proBNP | \* | \* |  | \* |  |
| Troponins | \* | \* |  | \* |  |
| CRP | \* |  |  |  | \* |
| IL-6 |  | \* |  |  |  |
| vWF |  | \* |  |  |  |
| D-dimer |  | \* |  |  |  |
| GDF-15 |  |  | \* | \* |  |
| TGF-β1 | \* |  |  |  | \* |
| Galectin-3 | \* |  |  |  | \* |
| sST2 | \* |  |  | \* | \* |
| LA size and function | \* | \* |  |  | \* |
| LAA morphology |  | \* |  |  |  |
| Myocardial fibrosis (MRI-LGE) |  | \* |  |  | \* |
| 4q25 locus | \* | \* |  |  |  |

\* An association between the biomarker and outcome has been identified in clinical trials. AF, atrial fibrillation; CRP, C-reactive protein;GDF-15, growth differentiation factor-15; IL-6, interleukin-6; LA, left atrium; LAA, left atrial appendage; MRI-LGE, magnetic resonance imaging-late enhanced gadolinium; NT-proBNP, N-terminal-pro B-type natriuretic peptide; sST2, soluble suppression of tumorigenicity 2; TGF-β1, transforming- growth factor-β1; vWF, von Willebrand factor.

******Figure 1. Schematic of biomarkers in atrial fibrillation.**

CRP, C-reactive protein; GDF-15, growth differentiation factor-15; IL-6, interleukin-6; LA, left atrium; LAA, left atrial appendage; MMPs, matrix metalloproteinases; NT-proBNP, N-terminal-pro B-type natriuretic peptide; sST2, soluble suppression of tumorigenicity 2; TGF-β1, transforming- growth factor-β1; vWF, von Willebrand factor.