Published in final edited form as:

Expert Rev Cardiovasc Ther. 2019 March ; 17(3): 193–207. doi:10.1080/14779072.2019.1562902.

Immunopathogenesis and biomarkers of recurrent atrial fibrillation following ablation therapy in patients with preexisting atrial fibrillation

John H Rosenberg¹, John H Werner¹, Gilman D Plitt¹, Victoria V Noble¹, Jordan T Spring¹, Brooke A Stephens¹, Aleem Siddique², Helenmari L Merritt-Genore², Michael J Moulton², and Devendra K Agrawal^{*,1}

¹Department of Clinical and Translational Science, Creighton University School of Medicine, Omaha, NE USA

²Department of Cardiothoracic Surgery, University of Nebraska Medical Center, Omaha, NE USA

Abstract

Introduction: Recurrent atrial fibrillation (RAF) following ablation therapy occurs in about 50% of patients. The pathogenesis of RAF is unknown, but is believed to be driven by atrial remodeling in the setting of background inflammation. Structural, electrophysiological and mechanical remodeling has been associated with atrial fibrillation (AF). Inflammation and fibrotic remodeling are the major factors perpetuating AF, as mediators released from the atrial tissues and cardiomyocytes due to mechanical and surgical injury could initiate the inflammatory process. In this article, we have critically reviewed the key mediators that may serve as potential biomarkers to predict RAF.

Areas covered: Damage associated molecular patterns, heat shock proteins, inflammatory cytokines, non-inflammatory markers, markers of inflammatory cell activity, and markers of collagen deposition and metabolism are evaluated as potential biomarkers with molecular treatment options in RAF.

Expert commentary: Establishing biomarkers to predict RAF could be useful in reducing morbidity and mortality. Investigations into the role of DAMPs participating in a sterile immune response may provide greater insight into the pathogenesis of RAF. Markers evaluating immune cell activity, collagen deposition, and levels of heat shock proteins show the greatest promise as potential biomarkers to predict RAF and develop novel therapies.

*Corresponding author: Devendra K Agrawal, Creighton University School of Medicine, CRISS II Room 510, 2500 California Plaza, Omaha, NE, 68178, USA, Tel: (402) 280 2938; Fax: (402) 280 1421, dkagr@creighton.edu.

Declaration of interest

The authors have no 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. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Keywords

Arrhythmia; Atrial Fibrillation; Danger associated molecular patterns; Fibrosis; Heat shock proteins; Inflammation; Surgical ablation

1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia seen in clinical practice, and it significantly contributes to morbidity, mortality, stroke, and peripheral embolism.^{1,2} The incidence of AF markedly increases with age, and its prevalence is expected to double in the next 50 years resultant to increases in longevity.²

One mode of managing AF is through catheter ablation. Current ablation techniques target areas of the atria susceptible to focal firing, re-entrant pathways, and areas that may house multiple propagating depolarizations.² The goal of ablation therapy is to eliminate the trigger that initiates fibrillation, such as a re-entrant pathway, or eliminate the area responsible for multiple depolarizations. Ablation therapy is effective for targeting electrophysiological changes but cannot adequately address general processes of remodeling. Currently, the most common areas for ablation are the pulmonary vein-left atrial junction, and the inferior vena cava- right atrial confluence.² Unfortunately, recurrent AF (RAF) following ablation therapy occurs in up to 50% of individuals.² Since the atrial fibrillation could be paroxysmal or intermittent AF and non-paroxysmal and persistent AF, the recurrence rate in paroxysmal AF is about 10-15% within one year, whereas the recurrence rate in non-paroxysmal AF is much higher.²⁻⁵ The mechanisms responsible for RAF may be different from the patients' original development of arrhythmia. The aim of this review is to highlight potential biomarkers of RAF following ablation therapy in patients with preexisting AF through the discussion of the pathogenesis of RAF, inflammatory cytokines, measures of inflammatory cell activity, and measures of collagen deposition and metabolism. By using these biomarkers for risk stratification of RAF, clinicians may be able to determine the utility of ablation surgery on a patient specific basis.

2. Pathogenesis of recurrent atrial fibrillation

Despite advances in surgical and medical management, the overall morbidity of AF has not changed significantly.¹ This is due to the incomplete understanding of the mechanisms driving AF. Structural, electrophysiological, and mechanical remodeling of atrial and ventricular myocardium has been associated with AF.⁶ The electrophysiological changes associated with AF include ectopic focal firing, re-entrant pathways shortening action potentials, and heterogenous impulse conduction caused by atrial fibrosis.⁶ Those changes work by disrupting refractory periods in atrial myocardium, resulting in calcium overload in cardiomyocytes. The cells respond to the calcium overload by decreasing expression of calcium L-type channels, which in turn decreases overall atrial contractile function.² The decrease of contractile function allows for atrial dilatation and structural remodeling. Left atrial structural remodeling leads to an increase of surface area. This allows for multiple impulses to propagate as opposed to a synchronized depolarization.² In addition to electrophysiologic changes, AF is also related to structural changes. Interstitial fibrosis is

associated with dilatation of the atria creating areas of heterogeneous composition. Fibrotic areas are interspersed between functioning cardiomyocytes. The heterogeneity of cells may disrupt conduction through the atria and cause AF to become increasingly persistent.² In fact, the development of atrial fibrosis is the hallmark of structural remodeling, and is considered to be the main mechanism perpetuating AF.⁶ Inflammation is central in the initiation of oxidative stress, fibrosis and structural remodeling, leading to thrombogenesis. Indeed, many mediators released from the atrial tissues and cardiomyocytes due to mechanical and surgical injury could initiate the inflammatory process. This could lead to fibrosis and structural remodeling that ultimately results in the reoccurrence of atrial fibrillation (Figure 1). However, it must be pointed that the inflammation could be significantly changed by different catheter ablation techniques. Indeed, there could be significant difference in the degree of atrial inflammation due to variable substrate modification by rotor ablation, stepwise ablation, complex fractionated atrial electrograms, ganglionated plexi ablation, and hybrid ablation. However, there is very limited information comparing these different ablation strategies. In the following sections, a few of the key mediators are discussed in relation to overall catheter ablation.

3. Inflammatory biomarkers in RAF

Various inflammatory biomarkers including damage-associated molecular patterns (DAMPs), heat shock proteins (HSPs), and inflammatory cytokines have been associated with RAF. Additional non-inflammatory markers such as anti-muscarinic receptor antibodies and troponin have also been associated with RAF. This section aims to review the role of high mobility group box-1 (HMGB-1), toll-like receptors (TLRs), inflammasomes, and HSPs along with inflammatory cytokines in pathogenesis of RAF and their role as possible biomarkers.

3.1 Damage associated molecular patterns

The pathogenesis of RAF has been associated with pre-existing inflammation.⁷ It is important to understand the difference between the development of new onset post-operative AF and recurrent post-operative AF. One measure that may differentiate between the two are DAMPs. In patients without underlying AF, the oxidative stress and ischemia caused by surgery activates a number of DAMPs, such as HMGB-1, mitochondrial DNA (mtDNA), TLRs, and HSPs. The activation of DAMPs, along with TLRs and inflammasome signaling results in an increase in pro-inflammatory cytokines with subsequent immune cell recruitment and activation. However, in patients with pre-existing AF, a baseline level of inflammation and remodeling exists, significantly increasing the risk of RAF following surgery. A prospective study by Sandler *et al.*⁸ in human subjects demonstrated increased release of mtDNA and corresponding TLR-9 activity following coronary bypass surgery. These increased levels of mtDNA and TLR-9 activity were associated with post-operative AF.⁸ The role of HMGB-1, TLRs, and the NLRP3 inflammasome in a sterile immune response in the setting of AF warrants further investigation.

3.2 High mobility group Box-1 in RAF

HMGB-1 is implicated in the pathogenesis and progression of atrial fibrillation. The serum level of HMGB-1 has been found to be increased in patients with AF.⁹ There is a strong relationship between HMGB-1 and inflammation.⁶ Cross-talk between HMGB-1 and inflammatory cytokines, and the association between oxidative stress and increased release of HMGB-1 has been well documented. An observational study by Wu *et al.*¹⁰ supported the association between oxidative stress and HMGB-1 release and further stipulated that inhibition of said oxidative stress may provide a therapeutic target for AF. The cross-talk between HMGB-1 and inflammatory cytokines such as tumor necrosis factor- α (TNF- α) has been well documented and can contribute to an inflammatory response through the recruitment of inflammatory cells.⁷ HMGB-1 may also play a role in remodeling, as expression of its receptors, receptor for advanced glycation end products (RAGE) and TLR-2, have been shown to be increased in patients with non-valvular AF.⁹ RAGE has been proposed to contribute to the pathogenesis of persistent AF. A study Lancefield *et al.*¹¹ showed that levels of soluble RAGE (sRAGE) and endogenous secretory RAGE (esRAGE) are able to predict the presence of persistent AF. More evidence is accumulating demonstrating the association between RAF and TLR activity following ablation therapy.

3.3 Toll-like receptors in RAF

TLRs have been shown to be increased in AF patients and associated with RAF following ablation therapy.¹² Gurses *et al.*¹² found in *in vivo* study in human subjects that left atrial expression of TLR-2, TLR-4, and serum levels of HMGB-1 were significantly increased in patients with persistent AF when compared to paroxysmal AF. Additionally, left atrial expression of TLR-2, TLR-4 and HMGB-1 were found to be significantly higher than peripheral levels within the patient population following cryoballoon ablation.¹² These localized findings support the notion that increased HMGB-1 and TLR activity may play a role in the pathogenesis and progression of RAF. Other studies by Gurses *et al.*¹³ investigated the levels of TLR expression on peripheral monocytes, and whether that expression could be used as an independent predictor of RAF following ablation therapy. The investigators found that monocyte TLR-2 and TLR-4 expression levels were higher in patients with AF, and monocyte TLR-4 expression levels was an independent predictor of RAF following cryoballoon ablation.¹³ This study highlights the possible role of TLR-mediated inflammation in the pathogenesis of AF, and may also provide a target for therapy.

3.4 Inflammasomes in AF

The NACHT, LRR and PYD domain containing protein-3 (NLRP3) inflammasome mediates caspase-1 activation and interleukin-1 β release in immune cells and has been associated with the pathogenesis of AF.¹⁴ An *in vitro* study assessing cardiomyocyte NLRP3 activity in human, canine and murine subjects found increased activity was linked to arrhythmogenesis.¹⁴ Specifically, this study found NLRP3 activity led to ectopic firing and AF susceptibility.¹⁴ These results also suggest inhibition of the NLRP3 inflammasome may serve as potential therapy to mitigate the electrophysiologic remodeling in AF. The study by Yao *et al.*¹⁴ suggested a novel role of the NLRP3 inflammasome in AF. The best known role of the NLRP3 inflammasome is its association with macrophages, IL-1 β secretion and

inflammation.¹⁵ The mechanistic link between NLRP3 inflammasome activation and electrophysiologic remodeling warrants further investigation and may serve as a potential biomarker to assess AF progression, in addition to its possible role as a therapeutic target.

3.5 Heat shock proteins (HSPs) in RAF

HSPs are a group of stress-response proteins heavily implicated in the development and progression of AF.^{16–20} In the normal stress response of a cell, HSPs work intracellularly as molecular chaperones to stabilize proteins, promote refolding of denatured proteins, and activate heat shock transcription factor 1 (HSF1) for degradation of more extensively damaged proteins. This helps establish a cytoprotective effect against oxidative stress, ischemic changes, and inflammation (Figure 2).^{20–22} Following stress activation in cardiomyocytes, HSPs have been shown to affect ion channels, calcium homeostasis, and the cytoskeleton. These combined effects prevent shortening of action potential duration, myolysis, contractile dysfunction, and protect against structural and electrophysiological cardiac remodeling known to lead to a number of disease processes.²⁰ While the role of HSPs in AF and RAF after ablation surgery is not fully understood, their protective role in cardiac remodeling has been widely studied, potentially preventing the progression and maintenance of AF rhythms.²³ For this reason, intracellular HSP levels, most notably HSP27, HSP70, and HSP60, provide valuable insight into a cell's ability to maintain sinus rhythm after surgery.

Unfortunately, measuring intracellular HSP levels require atrial tissue collection, making it an impractical option for routine assessment of post-ablation RAF. Serum HSP levels provide a more feasible alternative to intracellular levels, with the ability to noninvasively measure prior to surgical intervention; however, the correlation between intracellular and serum HSPs is not well understood. While studies have shown serum HSPs are markers of cellular damage after myocardial infarction due to leakage of intracellular contents, the relationship is less clear in AF.²⁴ As there is little cell necrosis in AF, it is difficult to ascertain if circulating HSPs are truly markers of cellular damage in a preoperative patient. Another study showed a cytokine-like role of HSP70 and HSP60 and suggested that HSPs may be secreted in response to stress and inflammation to provide an extracellular function.²⁵ Therefore, serum HSPs may be markers for a combination of cell damage, stress, inflammation, and others. Although the exact mechanism of HSP release in AF is not understood, some correlations between serum HSP levels and ablation outcome can still be drawn to serve as markers for overall risk of RAF.

3.5.1 HSP27—HSP27 has been shown to be the most promising HSP marker for post-ablation recurrence of AF. On a molecular level, HSP27 protects the cell against damage and electrophysiological changes through a number of mechanisms. HSP27 helps stabilize the actin cytoskeleton by directly binding to alpha-actinin and F-actin, maintaining the structural and contractile integrity of cardiomyocytes.²⁰ HSP27 also directly stabilizes L-type calcium channels, promoting normal electrical conduction through the cell. Although the exact mechanism is not fully understood, HSP27 is able to protect contractile proteins and ion channels against degradation from cysteine proteases, known to be activated during AF.²⁰ In addition, HSP27 is able to impede the proinflammatory TNF- α pathway, as well as increase

levels of anti-inflammatory cytokine IL-10, protecting against inflammation induced AF remodeling.¹⁷ Brundel *et al.*²⁶ showed that overexpression of HSP27 prior to subjecting human cardiomyocytes to a 10-fold rate increase successfully protected against tachypacing-induced myolysis. This supports that the additive molecular effects of HSP27 convey a significant survival benefit, minimizing cardiac remodeling secondary to myolysis. They also showed patients with paroxysmal AF had significantly higher levels of atrial intracellular HSP27 than in persistent AF patients.²⁶ All of these findings support the potential ability of HSP27 to protect against the progression of AF from paroxysmal to persistent type.

On a larger scale, several studies have attempted to correlate clinical outcomes with HSP27 levels. Hu *et al.*¹⁷ studied HSP27 in AF patients undergoing catheter ablation and found that preoperative levels of serum HSP27 were significantly lower in AF patients compared to their control counterparts, and lower in patients with persistent AF compared to patients with paroxysmal AF. Patients with low HSP27 serum levels were also more likely to have low atrial voltage, more non-pulmonary vein ectopies, and decreased mean fractionated interval.¹⁷ These findings support that high serum HSP27 levels are associated with decreased electrical remodeling lowering the likelihood of progression to sustained AF rhythms. Finally, Hu *et al.*¹⁷ found a negative correlation between serum HSP27 and AF recurrence at one year follow up after ablation. This particular finding supports the plausibility of using HSP27 as a marker for post-operative outcome in catheter ablation. However, this study is limited by the lack of information on intracellular HSP27 levels and expression, as the correlation between intracellular and serum HSP27 is not well established.

Cao *et al.*¹⁸ studied HSP27 expression and serum levels in AF patients undergoing mitral valve surgery, finding that atrial intracellular HSP27 expression was negatively correlated with apoptosis, myolysis, and interstitial fibrosis.¹⁸ While the intracellular HSP27 levels were reduced in patients with RAF after surgery, they were not an independent predictor of RAF. In addition, Cao *et al.*¹⁸ did not find a significant correlation between intracellular HSP27 expression and serum levels. Future studies will need to be done to fully establish the relationship between serum HSP27, intracellular HSP27, and clinical correlation. HSP27 continues to be of interest as a potential marker for AF outcomes after ablation.

3.5.2 HSP70—Historically, HSP70 has been primarily linked to ischemic heart disease and oxidative damage. HSP70 stabilizes myofibrils and ion channels in the setting of ischemic damage, leading to antiarrhythmic effects.^{20,22} HSP70 binds microtubules, minimizing myofibril disruption and protecting against contractile dysfunction. HSP70 has also been shown to stabilize potassium voltage gated ion channel proteins Kv1.5 and hERG (Kv11.1), maintaining the ultra-rapid delayed rectifier (I_{Kur}) and rapid delayed rectifier (I_{Kr}) potassium currents respectively, improving electrophysiological recovery during reperfusion, and decreasing the risk for arrhythmias.^{22,27} Similar to HSP27, intracellular HSP70 may have an anti-inflammatory effect, potentially decreasing levels of TNF- α and interleukin-6 (IL-6).¹⁸ On the other hand, one study found extracellular HSP70 to lose its cardioprotective effect and even adopt a pro-inflammatory role, directly resulting in increased TNF- α and IL-6.²⁵ Unfortunately, less information is available about HSP70's direct involvement in the pathophysiology of AF outside of the setting of ischemia and reperfusion.

One study done by Kornej *et al.*²⁸ looked at serum HSP70 and anti-HSP70 antibody levels prior to ablation and at 6 months follow up. However, neither were independently predictive of RAF. Interestingly, baseline anti-HSP70 antibody levels were significantly higher in persistent AF patients when compared to paroxysmal AF patients.²⁸ This may be due to a larger autoimmune response, resulting in increased inflammation, cardiac remodeling, and AF progression, but the exact role of anti-HSP antibodies in AF is not known. Other studies have looked at HSP70 as a marker for post-operative AF in other cardiac surgeries. Mandal *et al.*¹⁹ and Rammos *et al.*²⁹ found that atrial intracellular HSP70 expression was negatively correlated with rates of post-operative AF after coronary artery bypass grafting (CABG) and valve surgery, supporting a cardioprotective effect against ischemic and reperfusion states. These studies are less applicable to post-ablation surgery, as the patient population does not have AF prior to surgery.

Cao *et al.*¹⁸ investigated intracellular and serum HSP70 levels in patients with permanent AF undergoing mitral valve surgery. While serum HSP70 levels were not independent predictors of RAF after valve surgery, intracellular HSP70 levels were negatively correlated with several factors related to inflammation and remodeling, including TNF- α , IL-6, and apoptosis. Unlike HSP27, intracellular HSP70 did not demonstrate cardioprotective actions and had a stronger association with myolysis.¹⁸ This finding was supported by Brundel *et al.*²⁶ who found that overexpression of HSP70 was not sufficient to protect against tachypacing-induced myolysis. Additionally, intracellular HSP70 levels were not significantly correlated with serum HSP70 levels, reinforcing data from prior studies.^{18,19} No published studies have shown any value of serum HSP70 for predicting RAF after ablation or other cardiac surgery, and the lack of correlation to intracellular HSP70 levels further decreases the utility of HSP70 as a convenient biomarker. However, the negative relationship between intracellular HSP70 and inflammation, fibrosis, and electrical remodeling may provide a protective effect against the progression and maintenance of AF, and therefore warrants further investigation in a post-ablation setting.

3.5.3 HSP60—Much less is understood about HSP60 compared to HSP27 and HSP70, especially in the field of AF. HSP60 is known to contribute to mitochondrial integrity, maintaining the adenosine triphosphate (ATP) generation capacity of cardiomyocytes, especially in the presence of ischemic injury.²⁰ However, some evidence points to both intracellular and extracellular HSP60 having a deleterious effect thus promoting cell death and cardiac remodeling.^{18,30} Cao *et al.*¹⁸ found that intracellular and serum levels of HSP60 were positively correlated with apoptosis, interstitial fibrosis, inflammation, and recurrence of AF in AF patients undergoing mitral valve surgery. Kim *et al.*³⁰ investigated the effects of extracellular HSP60 and found that HSP60 directly activated TLR-4 and promoted apoptosis. Therefore, it is difficult to determine if HSP60 has a purely protective or deleterious effect in AF. Another study¹⁶ looked at data from the Multi-Ethnic Study of Atherosclerosis (MESA)²⁴ and was unable to find a correlation between baseline levels of HSP60 and the development of AF over 10-year follow up. However, this study was limited. Patients undergoing surgery, and intracellular HSP60 levels were not investigated limiting the ability to assess the association between HSP60 and AF. Anti-HSP60 antibodies also serve as a potential marker of interest, with one study finding a positive correlation between

serum anti-HSP60 antibody levels and increased rates of post-operative AF in patients undergoing a CABG.³¹ Currently, there are no published studies looking at HSP60 as a marker for RAF post-ablation. HSP60 continues to be of significant interest due to the strong correlation between intracellular and serum HSP60 levels, as well as its successful predictability of RAF after mitral valve surgery. Future investigation is warranted to fully elucidate the direct roles of intracellular and extracellular HSP60 in inflammation and cardiac remodeling, especially in the context of AF progression and recurrence.

3.6 Non-immune markers

Another possible marker that could be implemented in risk assessment for the recurrence of AF following ablation is high sensitivity cardiac troponin I (hs-cTnI). Previous studies have shown utility in using plasma hs-cTnI levels as a specific marker of cardiac damage.³² Reichlin *et al.*³³ found that total post-ablative hs-cTnI corresponds directly to the amount of energy delivered during ablation. A negative correlation between post-ablative levels of hs-cTnI and RAF suggests that lower levels of post-ablative hs-cTnI may indicate inadequate ablation.³⁴ Further studies are necessary to assess the effectiveness in trending hs-cTnI and predicting RAF both before and following ablation.

Anti-M2-muscarinic receptor autoantibodies (anti-M2-R) are implicated in a possible pathway leading to AF. Rabbits who were stimulated to produce anti-M2-Rs showed shorter atrial refractory periods and altered atrial electrophysiology.³⁵ In a human study of 75 patients with lone paroxysmal AF and 75 healthy subjects, anti-M2-R was significantly elevated in the AF group and was shown to be an independent predictor of AF.³⁶ Gurses *et al.*³⁷ showed pre-ablative serum anti-M2-R levels were correlated with the severity of atrial fibrosis and subsequent RAF following ablation. In another study of 76 AF patients, anti-M2-R serum levels were significantly higher than control groups. Likewise, patients with persistent AF had higher levels of serum anti-M2-R than those with paroxysmal AF. Pre-ablative anti-M2-R serum levels were found to be an independent predictor of RAF at one year.³⁸ Future studies could be improved by recording serum anti-M2-R before and after ablation to distinguish baseline levels from effects of ablation.

The neurohormone B type natriuretic peptide (BNP) and its precursor form N-terminal pro BNP (NT-proBNP) are secreted by cardiomyocytes in response to a variety of stimuli. A higher concentration of NT-pro BNP has been linked to occurrence of atrial fibrillation in patients with sinus rhythm.³⁹ BNP levels have been correlated with AF burden as well as recurrence after ablation with increasing BNP levels associated with increasing risk of arrhythmia recurrence.⁴⁰ This association of both BNP and NT-pro BNP with RAF after catheter ablation was confirmed in a meta-analysis.⁴¹

3.7 Inflammatory cytokines

While there are many contributors, the most significant inflammatory processes related to RAF are increases of inflammatory cytokines with migration of inflammatory cells and fibroblasts to the myocardium, promoting remodeling. Andrade *et al.*⁴² conducted a clinical trial to measure RAF following cryoballoon ablation, and found that early recurrence is strongly associated with late recurrence of AF. Early recurrence was defined as any

recurrence of AF greater than 30 seconds within the first three months following ablation therapy.⁴² Studies by Lim *et al.*⁴³ found the extent of the inflammatory response was predictive of early RAF following catheter ablation, but not late recurrence. Additionally, Al-Ani *et al.*⁴⁴ found increased inflammatory markers and inflammatory chemokines following catheter ablation therapy for AF in an *ex vivo* study. Al-Ani and colleagues hypothesized that targeting those chemokines may serve to reduce post-ablation activation of circulating leukocytes.⁴⁴

The presence of inflammatory cytokines including C-reactive protein (CRP), interleukin-2 (IL-2), IL-6, and TNF- α have been associated with AF.^{45–48} Cabrera-Bueno *et al.*⁴⁷ found increased serum levels of IL-2 were associated with RAF following catheter ablation. However, the causative role of inflammation in AF remains controversial.^{1,7} A meta-analysis published by Jiang *et al.*⁴⁹ noted an association between increased pre-ablative levels of CRP and IL-6, and RAF following radiofrequency ablation. This study also found increases of atrial natriuretic peptide (ANP), BNP, NT-pro-BNP, IL-6, CRP, low density lipoprotein (LDL), and tissue inhibitor of metalloproteinase-2 (TIMP-2).⁴⁹ The nonspecific findings described in the meta-analysis support a greater picture of general remodeling than a purely inflammatory one. Altun *et al.*⁵⁰ claimed increased levels of cardiotrophin-1, a cytokine in the IL-6 family, are associated with atrial remodeling in patients with persistent AF. Cardiotrophin-1 has been shown to promote structural changes in the heart through myocyte hypertrophy and collagen synthesis.⁵¹

An *in vivo* study of human subjects with a history of AF assessed the value of CRP and IL-6 as potential biomarkers to predict RAF.⁷ Masson *et al.*⁷ concluded from their study that inflammatory biomarkers may be raised in patients with a history of AF, but are at best weak predictors of recurrence. Similarly, Wen *et al.*⁵² assessed the safety and efficacy of catheter ablation therapy in patients with AF and rheumatoid arthritis and found no difference in RAF between patients with rheumatoid arthritis and controls. Rheumatoid arthritis has been associated with an increased incidence of AF, and increased pre-ablation serum levels of CRP were found in rheumatoid arthritis patients compared to controls.⁵² These results support the notion that inflammation is not a causative factor of RAF. A placebo controlled clinical trial by Sardu *et al.*⁵³ measured the effects of α -lipoic acid treatment on RAF following catheter ablation. Sardu and colleagues⁵³ found no difference in recurrence rates between the treated and controlled samples, but a significant reduction of inflammatory markers including CRP was found after 12 months.⁵⁴ Sugihara *et al.*⁵⁴ completed a similar *in vivo* study with human subjects by specifically inhibiting CRP expression through an antisense oligonucleotide. They found a significant decrease in CRP, but no reduction in AF.

A prospective study in human subjects suggested that inflammation is a consequence, rather than a cause of AF.⁵⁵ Kimura *et al.*⁴⁸ reported increased levels of TNF- α in jugular venous samples from patients with AF prior to ablation therapy compared to patients without a history of AF but significant for coronary artery disease. This finding is significant because it suggests a process other than ischemia increased TNF- α levels in AF. That same study showed increased TNF- α levels were also associated with persistent AF and positively correlated with left atrium size. Additionally, decreased TNF- α levels were found in patients following percutaneous mitral balloon valvuloplasty in patients with rheumatic mitral

stenosis with sinus rhythm.⁴⁸ Collectively, these studies suggest inflammatory cytokines may be signals of greater remodeling processes. Interestingly, decreased levels of CRP and IL-6 were found in patients who had successfully returned to normal sinus rhythm following catheter ablation for AF.⁵⁶ A reduction in inflammatory markers correlated with a return to normal sinus rhythm may signify a halt to arrhythmogenic remodeling processes. Inflammatory cytokines are a poor predictor of RAF due to their uncertain involvement in its pathogenesis. Recent studies suggest increased levels of inflammatory cytokines may be a consequence of AF and be more indicative of an underlying remodeling process. While the direct action of cytokines on cardiac remodeling does not appear to be strongly supported with recent data, the ability for cytokines to recruit inflammatory cells may play a role in RAF.

4. Inflammatory cells in RAF

The migration and activation of leukocytes has been strongly associated with AF.^{57–59} Smorodina *et al.*⁵⁷ found a moderate site-specific increase of inflammatory cells, monocytes prevailing, in the atrial myocardium of patients with AF. Chen *et al.*⁵⁸ found increased leukocyte infiltration in the atrial myocardium in patients with AF undergoing valve replacement surgery. Friedrichs *et al.*⁵⁹ found increased polymorphic neutrophils (PMN) infiltration in atrial myocardium following angiotensin II (ATII) administration in an *in vivo* study with murine models. Furthermore, the administration of angiotensin II increased the incidence of AF in murine models.⁵⁹ These studies associate leukocyte migration and the infiltration of atrial myocardium with AF, implying that inflammatory cells may serve to remodel the atria through subsequent fibrotic processes and contribute to AF persistence. Therefore, an increase of inflammation following ablation therapy may contribute to RAF through the infiltration of the atrial myocardium by inflammatory cells, and the subsequent fibrotic remodeling perpetuated by those cells.

4.1 Neutrophils infiltration in RAF

Atrial fibrosis has been associated with PMN infiltration of the atria.^{59,60} The pre-ablation neutrophil to lymphocyte ratio (NLR) in patients may be a reliable indicator of the potential for recurrence post-ablation. Canpolat *et al.*⁶¹ determined that the NLR is a reliable predictor of RAF following cryoablation. In this study, patients were separated into 4 quartiles depending on pre-ablation NLRs. It was found that those placed in the highest quartile had the greatest relapse rates, and those with the lowest NLR had the least amount of AF recurrence, with an overall sensitivity of 84% and a specificity of 75%.⁶¹ These results are promising, however, this study was done at a single center which may limit its application to the general population. Additionally, due to the nature of AF, many patients may have had asymptomatic episodes which could lead to underreporting of recurrence rates.

Although the Canpolat *et al.*⁶¹ study did not describe post-ablation NLR and its relation to AF recurrence, this relationship was studied by Guo *et al.*⁶² It was determined that post-ablation NLR was an independent risk factor for RAF.⁶² These findings support the predictive role of NLR in RAF. Taken together, these studies indicate that both pre-ablation and post-ablation NLR are useful in determining the risk of post-ablative AF recurrence.

However, comprehensive studies evaluating both pre- and post-ablation NLRs are necessary to determine the efficacy of its predictive value.

Studies in mice have associated atrial fibrosis with polymorphonuclear leukocytes (PMN) and fibroblast infiltration of the atrial tissue. PMNs were closely associated with inflamed atrial myocardium.⁵⁹ PMNs release myeloperoxidase (MPO) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the atrium, creating free radicals. Free radicals are created through nitric oxide (NO) uncoupling leading to atrial fibrosis and development of AF.^{59,63,64} The strong association of MPO and RAF was demonstrated by Li *et al.*⁶³ who found that patients with higher MPO levels were found to have higher risk of RAF following catheter ablation.

These results support the value of MPO as a predictor of RAF. MPO is a substance abundantly expressed by neutrophils, and increased MPO levels in RAF are consistent with increased PMN activity. The findings that both increased NLR and MPO levels may be predictive of RAF suggest that PMNs play a role in RAF pathogenesis. This process may be mediated by neutrophil release of MPO and subsequent fibrosis.

PMN extravasation and subsequent infiltration into atrial tissue is a necessary step of MPO-induced fibrosis.⁵⁹ Disruption of this process may reduce the risk for RAF due to decreased PMN-mediated fibrosis. Colchicine has been shown to reduce neutrophil chemotaxis.⁶⁵ A recent meta-analysis compared the effects of colchicine on post-operative incidence of AF to placebo or standard of care, it was found that patients who received colchicine had 30% less AF incidence.⁶⁶ Devereux *et al.*⁶⁷ demonstrated colchicine reduced early RAF following pulmonary vein isolation radiofrequency ablation. Colchicine treatment decreased RAF at 15 months without the use of antiarrhythmic drugs. It also demonstrated an improvement of quality of life measures at one year.⁶⁷ The ability of colchicine to reduce early RAF following catheter ablation gives credence to the idea that PMNs play an important role in RAF pathogenesis.

4.2 Monocytes infiltration in RAF

A second potential cellular marker to determine RAF risk is the monocyte-high density lipoprotein ratio (MHR). It was found that the pre-cryoablation MHR level was a good predictor of post-procedural RAF, regardless of whether the AF was paroxysmal, or persistent.⁶⁸ This study stratified patients into quartiles based on their pre-ablation MHR. They found MHR was positively correlated with RAF, although this study was limited by small sample size.⁶⁸ Monocyte activity may serve as a separate marker for AF recurrence. Gurses *et al.*¹³ investigated the relationship between toll-like receptor (TLR) expression on monocytes and RAF following ablation. TLR expression, specifically TLR4, was a significant predictor of RAF after cryoablation. Increased markers monocyte activity may be potential predictors of RAF. In addition to measuring the activity of leukocytes, measuring the levels of intracellular proteins may also have the potential to predict RAF.

5. Fibrotic remodeling in RAF

Atrial fibrosis has been shown to augment the recurrence rate following ablation therapy.⁶⁹ Recent studies focused on fibrotic remodeling of the heart highlight the role of transforming growth factor- β 1 (TGF- β 1). TGF- β 1 expression has been shown to increase in response to angiotensin II, and serves to activate fibroblasts to myofibroblasts and increase extracellular matrix (ECM) synthesis.¹ The relationship of TGF- β 1 levels and RAF has not been consistently established. Several prospective studies in human subjects have implicated TGF- β 1 as an independent predictor of RAF following ablation therapy.^{70–75} Additionally, a study by Cao *et al.*⁷⁶ demonstrated that polymorphisms in TGF- β 1 gene promoters may be useful in predicting the prognosis of patients in Chinese populations following ablative therapy for AF. Similar to TGF- β 1, matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) have been associated with AF, due to their role in ECM metabolism.^{1,2,73} Measuring fibrotic remodeling through the deposition and metabolism of the ECM may hold the most promise in predicting RAF. Markers of collagen deposition are emerging as possible predictive biomarkers for RAF.

RAF may be described as the consequence of atrial remodeling in the setting of background inflammation. Fibrotic remodeling of the atria is the most significant feature leading to AF perpetuation. Understanding the roles of the agents involved in the pathogenesis of RAF may allow for their use as biomarkers to predict RAF.

5.1 TGF- β 1

TGF- β 1 is a well-established profibrotic growth factor that plays a significant role as a mediator in atrial fibrosis.⁷⁷ Studies in murine models have shown constitutively active TGF- β 1 was associated with increased atrial fibrosis.^{78,79} TGF- β 1 induces collagen production and subsequent atrial fibrosis through the activation of cardiomyofibroblasts. TGF- β 1 propagates its signal via the Smad and TGF- β 1 activated kinase-1 (TAK1) signaling cascades. TGF- β 1 forms a complex with Smad2, Smad3, and Smad4 that translocates into the nucleus of cardiomyofibroblasts. This complex increases the transcription of genes encoding for type one collagen by binding to the promoter region.⁷⁹ TGF- β 1 may alternatively activate TAK1, which phosphorylates activated transcription factor 2 (ATF2) to form the Smad complex by a different mechanism. Once the Smad complex is formed, it acts in a similar manner by translocating into the nucleus and increasing the expression of genes associated with collagen synthesis. Direct activation of Smad by TGF- β 1 and alternative activation by TAK1 results in increased collagen deposition.⁷⁹

TGF- β 1 is a likely marker of collagen deposition and ongoing fibrosis, and has been found in increased levels in biopsies of patients with AF.^{49,77,79} Wu *et al.*⁷⁷ investigated the relationship between TGF- β 1 levels biopsied from the right atrial appendage and RAF following ablation therapy. Patients with increased TGF- β 1 levels were shown to have a significantly higher rate of RAF within one year.⁷⁷ Though limited by a small sample size, this study suggested a role for TGF- β 1 as a predictor of RAF. Further studies attempted to establish levels of peripheral TGF- β 1, opposed to local levels, as a predictor of RAF. A study of 200 patients undergoing catheter ablation for AF found that serum TGF- β 1 was an independent predictor of RAF in 46 patients with non-paroxysmal AF. However, no

significant correlation was reported in 154 patients with paroxysmal AF.⁷⁴ Tian *et al.*⁷⁰ found that serum TGF- β 1 concentrations prior to catheter ablation were predictive of RAF in a group of 38 patients with paroxysmal AF.⁷⁰ The findings of these two studies indicate a potential role of TGF- β 1 to predict RAF. However, these studies are limited by small sample size. Additionally, the conflicting findings in patients with paroxysmal AF warrant further investigation.

5.2 Fibroblasts

Fibroblasts contribute to AF development by promoting atrial fibrosis. Rapid sequential firing leads to the release of angiotensin II (ATII) and other mediators from cardiomyocytes. ATII signaling stimulates fibroblasts and cardiomyocytes to release TGF- β .⁸⁰ TGF- β 1 mediates the transformation of fibroblasts to myofibroblasts, enhancing the ability to deposit collagen.⁸¹ Fibrotic remodeling of the atria may create a source of ectopic electrical activity either through a re-entrant mechanism or through disruption of normal electrical architecture.⁸⁰

Fibrocytes, peripheral progenitor cells with the capability of differentiating into fibroblast and myofibroblasts, may play a role in the pathogenesis of AF.⁸² Liu *et al.*⁸³ recently reported increased levels of circulating fibrocytes in patients with persistent AF compared to paroxysmal, and between all forms of AF and healthy patients. The number of fibrocytes were predictive of RAF in patients with persistent AF. Additionally, fibrocytes in patients with persistent AF demonstrated enhanced ability to differentiate to myofibroblasts, in a process driven by TGF- β 1 expression.⁸³ Examining different cellular activity associated with AF may provide insights into its pathogenesis and ability to predict recurrence.

5.3 Collagen

Atrial fibrosis and its contribution to structural remodeling is an important component of AF pathogenesis (Figure 3).⁸⁴ Increased deposition of types I and III collagen and alterations to the ECM disrupt normal electrical conduction networks.^{6,85} Concentrations of serum markers of collagen deposition and breakdown including C-terminal propeptide of collagen type-I (CICP) and C-terminal telopeptide of collagen type-I (CITP) have been found to be higher in patients with AF than in healthy patients.⁸⁶ Additionally, the serum concentration of CICP was observed to be elevated in patients with persistent AF compared to those with paroxysmal AF, while CITP was not.⁸⁶ C-terminal peptide from procollagen-1 (PICP) and N-terminal peptide from procollagen III (PIINP) are other markers associated with left atrial fibrosis and development of AF in patients following cardiac surgery.⁸⁷

Several studies have reported that the degree of atrial remodeling, as measured by atrial diameter, indices of volume, and the duration of atrial conduction time, is predictive of RAF following cardioversion or ablation techniques.^{88–91} However, despite the role of collagen deposition in the pathogenesis of atrial remodeling and AF, the value of serum collagen turnover marker concentrations for predicting RAF following ablation procedures remains controversial.

Tian *et al.*⁷⁰ recently reported that preoperative serum PIINP levels were predictive, along with TGF- β , of RAF following catheter ablation for paroxysmal AF in 38 patients. Type IV

procollagen, however, was not predictive of recurrence.⁷⁰ Additionally, Okumura *et al.*⁹² also reported that elevated CITP (3.2 ± 1.1 ng/ml vs 2.7 ± 0.6 ng/ml) before catheter ablation predicted RAF.⁹² These findings seem to confirm the link between baseline serum collagen markers, atrial fibrosis, and RAF. However, this conclusion is challenged by the results of other studies.

Kimura *et al.*⁴⁸ analyzed serum biomarkers in 44 patients who underwent catheter ablation for AF. Samples were obtained before ablation and demonstrated no difference in CITP between patients who remained in sinus rhythm (3.5 ± 1 ng/ml) compared to those with RAF (3.5 ± 0.9 ng/ml). Levels of intact N-terminal propeptide of type 1 procollagen (PINP) were also not significantly predictive of RAF.⁴⁸ Begg *et al.*⁹³ recently reviewed several biomarkers including PIIINP and CITP collected from peripheral and intra-cardiac blood in 92 patients undergoing catheter ablation for AF. None of the markers tested significantly predicted RAF.⁹³

Many of the studies analyzing the predictive value of collagen turnover markers are limited by small sample size. Additionally, ablation performance at a single institution makes generalizing results of any single study to a broader population difficult. Further experimentation is needed to clarify the predictive value, or lack thereof, of serum levels of collagen markers including PIIINP and CITP for RAF.

5.4 MMP-2 and TIMP-2

MMP enzymes are a family of zinc-dependent endopeptidases that are directly involved in the degradation of matrix components between cells.⁷⁹ Abnormal increases in MMPs, such as those that occur secondary to increased levels of cytokines such as IL-6, may disrupt the balance of ECM metabolism. This may lead to fibrosis via increased matrix protein degradation and ECM turnover. Cardiac fibrosis and increased collagen levels have been associated with increased MMP activity.^{79,94,95} MMPs may be elevated secondary to generalized profibrotic states, though further characterization of this relationship is needed.^{79,94}

TIMPs balance MMPs by inhibiting their proteolytic activity.^{49,79,95} Elevated TIMP levels in select chambers of the heart have been associated with decreased fibrosis relative to areas of the heart without increased TIMP levels.⁹⁵

Multiple studies have analyzed the relationship between serum MMP and RAF. Sasaki *et al.*⁷³ observed that increased MMP-2 in 60 patients undergoing radiofrequency ablation predicted RAF.⁷³ Okumura *et al.*⁹² similarly found that increased MMP-2 predicted RAF in 50 patients following catheter ablation. Kimura *et al.*⁴⁸ also found that elevated MMP-2 at the time of ablation was correlated with RAF. While each of these studies is limited by small sample size and inconsistent methods reduce generalizability, MMP-2 is supported as a potentially useful predictor of RAF.

TIMP-2 has also been suggested as a predictor of RAF. Jiang *et al.*⁴⁹ found that increased serum TIMP-2 levels were associated with increased rates of early RAF. However, by three months following ablation, TIMP-2 levels were not associated with rates of recurrence.⁴⁹

Sasaki *et al.*⁷³ also found that increased TIMP-2 correlated with increased RAF. Interestingly, Kimura *et al.*⁴⁸ did not observe any significant relationship between TIMP-2 levels and RAF, casting some doubt on the reliability of TIMP-2 to predict RAF.

TGF- β 1 signaling, subsequent atrial collagen deposition, and the action of remodeling enzymes such as MMP-2 and TIMP-2 have been implicated in AF pathogenesis.^{77,84} Currently evidence on the efficacy of these different markers of collagen turnover to predict RAF is conflicted. It is possible that certain markers associated with collagen turnover such as TGF- β , MMP-2, and TIMP-2 may have prognostic utility for patients undergoing ablation for AF, while collagen products such as PIIINP and CITP do not. This suggests the possibility of independent mechanisms for promoting or predicting AF recurrence, or the inadequacy of sampling techniques.

6. Clinical predictors of recurrent atrial fibrillation

A number of clinical characteristics have been identified that are predictive of recurrence of atrial fibrillation. Winkle *et al.*⁹⁶ recently identified six independent variables that predicted RAF after ablation which they utilized to create a predictive scoring system. Excluding patients characteristics such as age and sex; atrial size and duration of atrial fibrillation have been most commonly correlated with the risk of recurrence likely reflecting chronicity of the disease with structural remodeling.⁹⁷ Interestingly NT-pro BNP has been correlated with both left atrial size and RAF after ablation providing a biochemical link between these clinical characteristics and ablation success.^{98–100} BNP/NT-pro BNP are non-specific neurohormones frequently associated with heart failure and It remains unclear if they play a mechanistic role in atrial fibrillation recurrence or are simply markers of the pathophysiologic processes in atrial fibrillation.

Left atrial size reduction after ablation has been correlated with changes in TGF- β 1 levels.¹⁰¹ TGF- β 1 levels have also been correlated with chronicity of the disease in the form of histologic atrial fibrosis.¹⁰² Unsurprisingly the ablation procedure itself impacts upon the serum levels of biomarkers and the cause vs effect relationship of these changes to recurrence of atrial fibrillation is unknown.¹⁰¹

The extent and mode of ablation are known to predict recurrence of atrial fibrillation.^{103,104} Intuitively one might expect more extensive ablation procedures to have a higher likelihood of success and this thought has been supported in a trial of different surgical ablation strategies.¹⁰⁵ Similarly the energy source and technique of ablation might also have an impact- although large head-to-head randomized trials are lacking a meta-analysis of studies seemed to favor radiofrequency ablation as a safe and effective modality for surgical ablation.¹⁰⁶ The efficacy of radiofrequency vs cryoablation is likely related to the extent of myocardial injury but also likely impacted by further technical and procedural details such as the duration of ablation.^{106–108} The impact of these ablation modalities upon biomarkers have not been conclusively studied.

Biomarkers that are specific to atrial fibrillation and correlate with patient characteristics and likelihood of ablation success are currently lacking. Could a biomarker be used to predict the

likelihood of recurrence prior to an ablation (whether catheter based or surgical) and might this in turn be utilized to guide the timing, modality and extent of ablation? Could the response of that same biomarker after ablation then be evidence of treatment success or predictive of future treatment failure and hence continue to guide ongoing therapy? Such a biomarker would be ideal in the treatment of atrial fibrillation.

7. Molecular treatment options

Biomarkers may be used to both assess the risk of RAF and track its progression. Many biomarkers are associated with the pathogenesis of RAF and could therefore present as possible therapeutic targets to mitigate RAF.

7.1 HSP induction therapy

While HSPs may serve as useful serum markers for predicting post-ablation AF, the intentional induction of HSPs prior to surgery may provide a cardioprotective effect and lower rates of AF recurrence. Geranylgeranylacetone (GGA) is a nontoxic pharmaceutical that has been shown to successfully induce HSP synthesis in the myocardium as well as other tissues.^{23,109} Administration of GGA to rabbits undergoing heart failure due to coronary artery ligation was able to induce transcriptional activation of HSPs, including HSP27 and HSP70, and protect against electric remodeling of the heart.¹⁰⁹ Specifically, rabbits given GGA showed higher expression of the following calcium, sodium, and potassium ion channel proteins: Cav1.2 (I_{Ca-L}), Nav1.5 (I_{Na}), Kir2.1 (I_{K1}), Kv1.4 (I_{to}), Kv7.1 (I_{Ks}), and Kv11.1 (I_{Kr}), as well as sarcoplasmic reticulum Ca^{2+} -ATPase.¹⁰⁹ As a result, these rabbits had less action potential duration dispersion, decreased triggered activity, and decreased inducibility of AF when compared to rabbits with heart failure not given GGA.¹⁰⁹ Overall, this study supports that the induction of HSPs could protect against electric remodeling, thereby lowering the likelihood of AF induction. However, this study was done in a heart failure setting, making it less applicable to post ablation in healthy patients. A new prospective study underway, called the HALT&REVERSE project, is investigating the serum and intracellular HSP levels, including HSP27 and HSP70, in patients undergoing cardiac surgery, pulmonary vein isolation, or electrical cardioversion.¹¹⁰ While this project aims to further characterize the diagnostic and predictable capabilities of HSPs in post-operative AF, it has the long-term goal to investigate the use of GGA and HSP inducers to halt and reverse the progression of AF.^{21,110} Further investigation is needed to determine the efficacy and feasibility of HSP induction in decreasing RAF post-ablation. In addition to HSPs, other systemic inflammatory markers have been associated with AF.

7.2 DAMP inhibition therapy

There is little data on DAMP inhibition specific to the heart. Other studies have demonstrated that inhibition of HMGB-1 or the HMGB-1 and RAGE complex may have therapeutic effects.¹¹¹ Agents including the HMGB-1 antagonist, recombinant Box A, and the small molecular inhibitor of HMGB-1 derived from glycyrrhizin were shown to achieve therapeutic effects mitigating inflammation *in vivo* and *in vitro* studies.¹¹¹ NLRP3 inhibition has been suggested as a possible therapy in RAF. No current study investigates NLRP3 inhibition in the setting of RAF. However, the CANTOS clinical trial is currently

investigating whether canakinumab, an IL-1 β and CRP inhibitor, may reduce recurrent myocardial infarctions and provide a novel cytokine-based therapy.¹¹² NLRP3 is a potent activator of IL-1 β , and inhibition of that interleukin may mitigate the downstream inflammatory effects of the inflammasome. There is no currently suggested therapy to mitigate the possible electrophysiologic remodeling associated with atrial NLRP3 activity. Other therapies investigating the role of statins in preventing RAF, antiarrhythmic drugs, and secondary catheter ablation have shown repeat ablation is the most effective.^{113,114}

7.3 Anti-arrhythmic therapy

Antiarrhythmic drug therapy is frequently utilized as the first treatment modality for patients with atrial fibrillation. Each drug has its unique side effect profile and drug therapy is frequently individualized based on patient characteristics, treatment goals and side effects.^{115,116}

One of the goals of ablation therapy is to reduce the need for these medications, however, they may have a role in the prevention of RAF after ablation. Two recent meta-analyses concluded that anti-arrhythmic drug use after catheter ablation for atrial fibrillation reduced early arrhythmia recurrence but did not prevent late recurrence of atrial arrhythmias.^{117,118} A randomized trial of anti-arrhythmic drug treatment after surgical ablation demonstrated reduced early recurrence of atrial arrhythmias in the treatment arm.¹¹⁹ Any correlation between post-ablation recurrence, drug therapy and biomarkers is not well elucidated.

8. Conclusion

Treatment strategies for patients with AF include medical and interventional management. Invasive ablation techniques aim to restore normal sinus rhythm by eliminating aberrant conduction pathways. However, these modalities are plagued by high levels of recurrence. The ability to predict patients at a higher risk of RAF would help identify the best surgical candidates, potentially sparing patients the need for invasive procedures that are not likely to be successful. Additionally, identification of patients with increased likelihood of RAF could tailor surveillance for recurrence allowing prompt recognition and treatment, reducing progression of recurrent arrhythmia. The identification of markers predictive of RAF would improve current treatment for AF. This review highlighted several markers that show promise as potential predictive biomarkers for RAF. The most promising markers include ratios of inflammatory cell activity, levels of circulating fibrocytes, TGF- β 1, markers of collagen deposition, and heat shock proteins. Further studies investigating those markers may provide a tenable biomarker.

9. Expert commentary

Recent studies have sought to identify serum markers predictive of RAF by investigating factors associated with the presence and pathogenesis of AF. A sterile immune response seems to be the predominant mode of inflammation influencing AF. Molecules such as DAMPs and HSPs represent an area of great potential for prediction of RAF. Specifically, HSP27, HSP70, and HSP60 have been identified as potentially useful. However, as these proteins are normally intracellular, sampling techniques remain a challenge. Current

techniques rely on serum detection of extracellular HSPs released following severe cellular stress, or their antibodies. However, the correlation of intracellular to extracellular HSP levels is not established and should be further analyzed. Further efforts should focus on HSP27 and HSP60. HSP27 has demonstrated promise as a predictor of RAF, and HSP60 has demonstrated a stronger correlation between intracellular and serum levels. The correlation between intracellular and extracellular levels of HSP60 raises its practicality for use as a potential biomarker. The focus on HSP27 and HSP60 may come at the expense of HSP70, which despite adequate interest has failed to demonstrate predictive value.

10. Five-year view

Further investigations into the electrophysiologic remodeling potential of the NLRP3 inflammasome may yield promising results. Additional investigations into HMGB-1 signaling with TLRs and RAGE may also provide insight into the pathogenesis of RAF, though due to their ubiquitous nature, are not likely to be specific biomarkers.

A significant number of studies have focused on serum levels of inflammatory cytokines. IL-2, IL-6, CRP, and TNF- α have so far been shown to be nonspecific and unreliable as predictors of RAF. Further research in this subject should aim to track serum levels before and after ablation and to monitor progression.

Other promising serum factors assessed for predictive utility include hs-cTnI and anti-M2-R. Studies so far remain limited and definitive conclusions cannot be drawn, requiring further research.

Markers for atrial infiltration by several cell types also warrant further investigation. Attempts should be made to replicate the recent findings that levels of circulating fibrocytes predict RAF. Additionally, NLR and MHR, markers of neutrophil and monocyte infiltration and activity within the atria, are promising as potential cost-efficient modalities for predicting RAF. Increased attention should be paid to the replicating these results and establishing the predictive power of these markers. As with other subjects, the development of local sampling strategies could improve the specificity of findings to atrial involvement and are of the highest priority.

Serum markers involved in atrial fibrosis including TGF- β 1, collagen breakdown products, MMP-2, and TIMP-2 have demonstrated encouraging early results as potential predictors of RAF. TGF- β 1 and MMP-2 have demonstrated especially intriguing potential. The currently inconclusive results analyzing collagen breakdown products warrant further study. Future studies require greater patient numbers and enhanced local sampling techniques to measure collagen breakdown products to provide more conclusive results.

Attempts to identify reliable predictors of RAF have faced similar challenges that future studies need to address in the next five years. Generally, many studies were performed at a single institution, and limited by small sample sizes, making generalization difficult. Study populations are often not comparable due to differing baseline characteristics amongst factors including AF classification as paroxysmal or persistent. Larger multicenter studies would improve the interpretation and strength of future findings. Serum marker collection is

also a somewhat nonspecific process, limiting the specificity of many markers to atrial pathogenesis. Development of local sampling would improve this predictive power. Finally, measurement of marker levels before ablation and subsequent marker trending could help identify patients likely to develop RAF before overt clinical symptoms develop. In the next five years, these steps should establish useful markers, as well as more definitively identify which markers do not offer clinical utility. This could help narrow the focus of time and resources on more promising markers.

Acknowledgements

The content of this review article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Funding

D K Agrawal is supported by research grants R01HL112597, R01HL116042, R01 HL120659, and R01HL144125 from the National Institutes of Health, USA.

References

Papers of special note have been highlighted as:

* of interest

** of considerable interest

1. Turagam MK, Mirza M, Werner PH, et al. Circulating Biomarkers Predictive of Postoperative Atrial Fibrillation. doi:10.1097/CRD.0000000000000059.
2. Vizzardi E, Curnis A, Latini MG, et al. Risk factors for atrial fibrillation recurrence. *J Cardiovasc Med.* 2014;15(3):235–253. doi:10.2459/JCM.0b013e328358554b.*This review highlighted several cytokines and pathways associated with recurrent atrial fibrillation.
3. Chao T-F, Tsao H-M, Lin Y-J, et al. Clinical outcome of catheter ablation in patients with nonparoxysmal atrial fibrillation: results of 3-year follow-up. *Circ Arrhythm Electrophysiol.* 2012; 5(3): 514–520. [PubMed: 22550126]
4. Nielsen JC, Johannessen A, Raatikainen P, et al. Long-term efficacy of catheter ablation as first-line therapy for paroxysmal atrial fibrillation: 5-year outcome in a randomised clinical trial. *Heart* 2017; 103(5): 368–376. [PubMed: 27566295]
5. Padfield GJ, Steinberg C, Swampillai J, et al. Progression of paroxysmal to persistent atrial fibrillation: 10-year follow-up in the Canadian Registry of Atrial Fibrillation. *Heart Rhythm* 2017; 14(6): 801–807. [PubMed: 28232263]
6. Dzeshka MS, Lip GYH, Snezhitskiy V, Shantsila E. THE PRESENT AND FUTURE Cardiac Fibrosis in Patients With Atrial Fibrillation Mechanisms and Clinical Implications. 2015. doi: 10.1016/j.jacc.2015.06.1313.
7. Masson S, Aleksova A, Favero C, et al. Predicting atrial fibrillation recurrence with circulating inflammatory markers in patients in sinus rhythm at high risk for atrial fibrillation: data from the GISSI atrial fibrillation trial. *Heart* 2010; 96(23): 1909–1914. doi:10.1136/hrt.2009.191460. [PubMed: 20965978] *This paper demonstrated circulatory inflammatory markers were unreliable predictors of atrial fibrillation recurrence.
8. Sandler N, Kaczmarek E, Itagaki K, et al. Mitochondrial DAMPs Are Released During Cardiopulmonary Bypass Surgery and Are Associated With Postoperative Atrial Fibrillation. *Hear Lung Circ.* 2018;27(1):122–129. doi:10.1016/j.hlc.2017.02.014.
9. Hu X-R, Wang X-H, Liu H-F, Zhou W-J, Jiang H. High mobility group box 1 protein: possible pathogenic link to atrial fibrillation. *Chin Med J (Engl).* 2012;125(13):2346–2348. <http://www.ncbi.nlm.nih.gov/pubmed/22882860>. Accessed August 17, 2018. [PubMed: 22882860]

10. Wu Y, Zhang K, Zhao L, Guo J, Hu X, Chen Z. Increased serum HMGB1 is related to oxidative stress in patients with atrial fibrillation. *J Int Med Res.* 2013;41(6):1796–1802. doi: 10.1177/0300060513503917. [PubMed: 24265331]
11. Lancefield TF, Patel SK, Freeman M, et al. The Receptor for Advanced Glycation End Products (RAGE) Is Associated with Persistent Atrial Fibrillation. Oury TD, ed. *PLoS One.* 2016;11(9):e0161715. doi:10.1371/journal.pone.0161715. [PubMed: 27627677]
12. Gurses KM, Kocyigit D, Yalcin MU, et al. Platelet Toll-like receptor and its ligand HMGB-1 expression is increased in the left atrium of atrial fibrillation patients. *Cytokine.* 2018;103:50–56. doi:10.1016/j.cyto.2017.12.007. [PubMed: 29324261]
13. Gurses KM, Kocyigit D, Yalcin MU, et al. Monocyte Toll-Like Receptor Expression in Patients with Atrial Fibrillation. *Am J Cardiol.* 2016;117(9):1463–1467. doi:10.1016/j.amjcard.2016.02.014. [PubMed: 26988292]
14. Yao C, Veleva T, Scott L, et al. Enhanced Cardiomyocyte NLRP3 Inflammasome Signaling Promotes Atrial Fibrillation. *Circulation.* 5 2018:CIRCULATIONAHA.118.035202. doi:10.1161/CIRCULATIONAHA.118.035202.
15. He G, Tan W, Wang B, et al. Increased M1 Macrophages Infiltration Is Associated with Thrombogenesis in Rheumatic Mitral Stenosis Patients with Atrial Fibrillation. Lai H-C, ed. *PLoS One.* 2016;11(3):e0149910. doi:10.1371/journal.pone.0149910. [PubMed: 26930272]
16. Maan A, Jorgensen NW, Mansour M, et al. Association between Heat Shock Protein-60 and Development of Atrial Fibrillation: Results from the Multi-Ethnic Study of Atherosclerosis (MESA). *Pacing Clin Electrophysiol.* 2016;39(12):1373–1378. doi:10.1111/pace.12969. [PubMed: 27807875]
17. Hu Y-F, Yeh H-I, Tsao H-M, et al. Electrophysiological Correlation and Prognostic Impact of Heat Shock Protein 27 in Atrial Fibrillation. *Circ Arrhythmia Electrophysiol.* 2012;5(2):334–340. doi: 10.1161/CIRCEP.111.965996. **This reference provides direct evidence that patients who developed recurrent post-ablation AF had significantly decreased serum HSP27 levels. This suggests that HSP27 may be a useful biomarker for predicting post-operative prognosis.
18. Cao H, Xue L, Xu X, et al. Heat shock proteins in stabilization of spontaneously restored sinus rhythm in permanent atrial fibrillation patients after mitral valve surgery. *Cell Stress Chaperones.* 2011;16(5):517–528. doi:10.1007/s12192-011-0263-9. [PubMed: 21455828] *While this reference does not study patients who underwent ablation for AF, it does provide significant information about HSPs of interest in the context of preventing AF recurrence after surgery. Specifically, this reference provides information about HSP27, 70, and 60, and their relationship with apoptosis, TNF-alpha, IL-6 levels, myolysis, and AF recurrence, as well as relating serum to intracellular HSP levels.
19. Mandal K, Torsney E, Poloniecki J, Camm AJ, Xu Q, Jahangiri M. Association of high intracellular, but not serum, heat shock protein 70 with postoperative atrial fibrillation. *Ann Thorac Surg.* 2005;79(3):865–871. doi:10.1016/j.athoracsur.2004.08.018. [PubMed: 15734396]
20. Brundel BJM, Ke L, Dijkhuis A-J, et al. Heat shock proteins as molecular targets for intervention in atrial fibrillation. *Cardiovasc Res.* 2008;78(3):422–428. doi:10.1093/cvr/cvn060. [PubMed: 18326558]
21. Lanter EAH, van Marion DMS, Steen H, de Groot NMS, Brundel BJM. The future of atrial fibrillation therapy: Intervention on heat shock proteins influencing electropathology is the next in line. *Netherlands Hear J.* 2015;23(6):327–333. doi:10.1007/s12471-015-0699-0.
22. Min TJ, Jo WM, Shin SY, Lim HE. The protective effect of heat shock protein 70 (Hsp70) in atrial fibrillation in various cardiomyopathy conditions. *Hear Vessel.* 2015;30(3):379–385. doi:10.1007/s00380-014-0521-8.
23. Van Marion D, Lanter EA, Wiersma M, et al. Diagnosis and Therapy of Atrial Fibrillation: the Past ,the Present and the Future. *J Atr Fibrillation* 2015;8(2): 1216. [PubMed: 27957185]
24. Bild DE, Bluemke DA, Burke GL, et al. Multi-Ethnic Study of Atherosclerosis: Objectives and design. *Am J Epidemiol.* 2002;156(9):871–881. doi:10.1093/aje/kwf113. [PubMed: 12397006]
25. Asea A, Kraeft SK, Kurt-Jones EA, et al. HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nat Med.* 2000;6(4): 435–442. doi:10.1038/74697. [PubMed: 10742151]

26. Brundel BJJM, Henning RH, Ke L, van Gelder IC, Crijns HJGM, Kampinga HH. Heat shock protein upregulation protects against pacing-induced myolysis in HL-1 atrial myocytes and in human atrial fibrillation. *J Mol Cell Cardiol.* 2006;41(3):555–562. doi:10.1016/j.yjmcc.2006.06.068. [PubMed: 16876820] *This reference provides evidence that induction of HSP27 was sufficient to protect against tachypacing-induced myolysis whereas HSP70 was not sufficient. This suggests HSP27 may have a more direct role in protecting against the progression and maintenance of AF.
27. Li P, Kurata Y, Maharani N, Mahati E, Higaki K, Hasegawa A. E3 ligase CHIP and Hsc70 regulate Kv1.5 protein expression and function in mammalian cells. *J Mol Cell Cardiol.* 2015;86:138–146. [PubMed: 26232501]
28. Kornej J, Reinhardt C, Kosiuk J, et al. Response of circulating heat shock protein 70 and anti-heat shock protein 70 antibodies to catheter ablation of atrial fibrillation. *J Transl Med.* 2013;11(1):49. doi:10.1186/1479-5876-11-49. [PubMed: 23432758]
29. Rammos K, Koullias GJ, Hassan MO, et al. Low Preoperative HSP70 Atrial Myocardial Levels Correlate Significantly with High Incidence of Postoperative Atrial Fibrillation after Cardiac Surgery. *Vascular.* 2002;10(3):228–232. doi:10.1177/096721090201000309.
30. Kim S, Stice J, Chen L, Jung J. Extracellular heat shock protein 60, cardiac myocytes, and apoptosis. *Circulation.* 2009;105(12):1186–1195. doi:10.1161/CIRCRESAHA.109.209643.
31. Oc M, Ucar HI, Pinar A, et al. Heat shock protein 60 antibody. A new marker for subsequent atrial fibrillation development. *Saudi Med J.* 2007;28(6):844–847. doi:20061166' [pii]. [PubMed: 17530097]
32. Segraves JM, Frishman WH. Highly Sensitive Cardiac Troponin Assays. *Cardiol Rev.* 2015;23(6):1. doi:10.1097/CRD.000000000000087. [PubMed: 25275716]
33. Reichlin T, Lockwood SJ, Conrad MJ, et al. Early release of high-sensitive cardiac troponin during complex catheter ablation for ventricular tachycardia and atrial fibrillation. *J Interv Card Electrophysiol.* 2016;47(1):69–74. doi:10.1007/s10840-016-0125-6. [PubMed: 26971332]
34. Aksu T, Golcuk SE, Guler TE, Yalin K, Erden I. Prediction of mid-term outcome after cryo-balloon ablation of atrial fibrillation using post-procedure high-sensitivity troponin level. *Cardiovasc J Afr.* 2015;26(4):165–170. doi:10.5830/CVJA-2015-027. [PubMed: 25783791]
35. Hong CM, Zheng QS, Liu XT, Shang FJ, Wang HT, Jiang WR. Effects of Autoantibodies against M2 Muscarinic Acetylcholine Receptors on Rabbit Atria in vivo. *Cardiology.* 2009;112(3):180–187. doi:10.1159/000149152. [PubMed: 18679026]
36. YALCIN MU, GURSES KM, KOCYIGIT D, et al. Cardiac Autoantibody Levels Predict Recurrence Following Cryoballoon-Based Pulmonary Vein Isolation in Paroxysmal Atrial Fibrillation Patients. *J Cardiovasc Electrophysiol.* 2015;26(6):615–621. doi:10.1111/jce.12665. [PubMed: 25788224]
37. Gurses KM, Yalcin MU, Kocyigit D, et al. M2-muscarinic acetylcholine receptor autoantibody levels predict left atrial fibrosis severity in paroxysmal lone atrial fibrillation patients undergoing cryoablation. *Europace.* 2015;17(2):239–246. doi:10.1093/europace/euu228. [PubMed: 25238749]
38. Zou C, Zhang Z, Zhao W, et al. Predictive value of pre-procedural autoantibodies against M2-muscarinic acetylcholine receptor for recurrence of atrial fibrillation one year after radiofrequency catheter ablation. *J Transl Med.* 2013;11(1):7. doi:10.1186/1479-5876-11-7. [PubMed: 23294565]
39. Staszewsky L, Masson S, Barlera S, et al. On the behalf of the GISSI-AF Investigators. Cardiac Remodeling, Circulating Biomarkers and Clinical Events in Patients with a History of Atrial Fibrillation. Data from the GISSI-AF Trial. *Cardiovasc Drugs Ther.* 2015 12;29(6):551–561. [PubMed: 26546322]
40. Hussein AA, Saliba WI, Martin DO, et al. Plasma B-type natriuretic peptide levels and recurrent arrhythmia after successful ablation of lone atrial fibrillation. *Circulation.* 2011 5 17;123(19):2077–82. [PubMed: 21536999]
41. Zhang Y, Chen A, Song L, Li M, Chen Y, He B. Association Between Baseline Natriuretic Peptides and Atrial Fibrillation Recurrence After Catheter Ablation. *Int Heart J.* 2016;57(2): 183–9. [PubMed: 26973277]
42. Andrade JG, Khairy P, Macle L, et al. Incidence and Significance of Early Recurrences of Atrial Fibrillation After Cryoballoon Ablation: Insights From the Multicenter Sustained Treatment of

- Paroxysmal Atrial Fibrillation (STOP AF) Trial. *Circ Arrhythmia Electrophysiol.* 2014;7(1):69–75. doi:10.1161/CIRCEP.113.000586.
43. Lim HS, Schultz C, Dang J, et al. Time course of inflammation, myocardial injury, and prothrombotic response after radiofrequency catheter ablation for atrial fibrillation. *Circ Arrhythm Electrophysiol.* 2014;7(1):83–89. doi:10.1161/CIRCEP.113.000876. [PubMed: 24446024] **This study found TGF- β 1 and PIIINP were predictive of atrial fibrillation recurrence.
 44. Al-Ani M, Ambadapadi S, Yaron JR, et al. Atrial Fibrillation Ablation Increases Inflammation-Chemokine Modulation Suppresses Activation of Leukocytes Isolated after Ablation. *Cardiovasc Hematol Disord Targets.* 2018;17(3):195–204. doi:10.2174/1871529X17666170918140442.
 45. Issac TT, Dokainish H, Lakkis NM. Role of Inflammation in Initiation and Perpetuation of Atrial Fibrillation: A Systematic Review of the Published Data. *J Am Coll Cardiol.* 2007;50(21):2021–2028. doi:10.1016/J.JACC.2007.06.054. [PubMed: 18021867]
 46. Guo Y, Lip GYH, Apostolakis S. Inflammation in Atrial Fibrillation. *J Am Coll Cardiol.* 2012;60(22):2263–2270. doi:10.1016/J.JACC.2012.04.063. [PubMed: 23194937]
 47. Cabrera-Bueno F, Medina-Palomo C, Ruiz-Salas A, et al. Serum levels of interleukin-2 predict the recurrence of atrial fibrillation after pulmonary vein ablation. *Cytokine.* 2015;73(1):74–78. doi: 10.1016/j.cyto.2015.01.026. [PubMed: 25743240]
 48. Kimura T, Takatsuki S, Inagawa K, et al. Serum Inflammation Markers Predicting Successful Initial Catheter Ablation for Atrial Fibrillation. *Hear Lung Circ.* 2014;23:636–643. doi:10.1016/j.hlc.2014.02.003.*This paper described the role of MMPs and TNF- α following catheter ablation.
 49. Jiang H, Wang W, Wang C, Xie X, Hou Y. Association of pre-ablation level of potential blood markers with atrial fibrillation recurrence after catheter ablation: A meta-analysis. *Europace.* 2017;19(3):392–400. doi:10.1093/europace/euw088. [PubMed: 27386883]
 50. Altun I, Pamukcu B, Yildiz CE, et al. Cardiotrophin-1: A new predictor of atrial fibrillation relapses after successful cardioversion. *Bosn J Basic Med Sci.* 2015;15(3):68–73. doi:10.17305/bjbm.2015.503. [PubMed: 26295297]
 51. Calabrò P, Limongelli G, Riegler L, et al. Novel insights into the role of cardiotrophin-1 in cardiovascular diseases. *J Mol Cell Cardiol.* 2009;46(2):142–148. doi:10.1016/j.yjmcc.2008.11.002. [PubMed: 19059413]
 52. Wen SN, Liu N, Li SN, et al. Catheter ablation of atrial fibrillation in patients with rheumatoid arthritis. *J Cardiol.* 2015;66(4):320–325. doi:10.1016/j.jjcc.2014.12.003. [PubMed: 25583089]
 53. Sardu C, Santulli G, Santamaria M, et al. Effects of Alpha Lipoic Acid on Multiple Cytokines and Biomarkers and Recurrence of Atrial Fibrillation Within 1 Year of Catheter Ablation. *Am J Cardiol.* 2017;119(9):1382–1386. doi:10.1016/j.amjcard.2017.01.040. [PubMed: 28258730]
 54. Sugihara C, Freemantle N, Hughes SG, Furniss S, Sulke N. The effect of C-reactive protein reduction with a highly specific antisense oligonucleotide on atrial fibrillation assessed using beat-to-beat pacemaker Holter follow-up. <https://link.springer-com.cuhsl.creighton.edu/content/pdf/10.1007%2Fs10840-015-9986-3.pdf>. Accessed May 25, 2018.
 55. Kallergis EM, 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(2):200–204. doi:10.1136/hrt.2006.108688. [PubMed: 17575330]
 56. Osmancik P, Peroutka Z, Budera P, Herman D, Stros P, Straka Z. Changes in cytokine concentrations following successful ablation of atrial fibrillation. *Eur Cytokine Netw.* 2010;21(4):278–284. doi:10.1684/ecn.2010.0216. [PubMed: 21106468]
 57. Smorodinova N, Bláha M, Melenovský V, et al. Analysis of immune cell populations in atrial myocardium of patients with atrial fibrillation or sinus rhythm. Vinci MC, ed. *PLoS One.* 2017;12(2):e0172691. doi:10.1371/journal.pone.0172691. [PubMed: 28225836]
 58. Chen M-C, Chang J-P, Liu W-H, et al. Increased Inflammatory Cell Infiltration in the Atrial Myocardium of Patients With Atrial Fibrillation. *Am J Cardiol.* 2008;102(7):861–865. doi: 10.1016/J.AMJCARD.2008.05.038. [PubMed: 18805111]
 59. Friedrichs K, Adam M, Remane L, et al. Induction of Atrial Fibrillation by Neutrophils Critically Depends on CD11b/CD18 Integrins. Sovari AA, ed. *PLoS One.* 2014;9(2):e89307. doi:10.1371/journal.pone.0089307. [PubMed: 24558493]

60. Frustaci A, Chimenti C, Bellocci F, Morgante E, Russo M, Maseri A. Histological substrate of atrial biopsies in patients with lone atrial fibrillation. *Circulation*. 1997;96(4):1180–1184. [PubMed: 9286947]
61. Canpolat U, Aytemir K, Yorgun H, et al. Role of preablation neutrophil/lymphocyte ratio on outcomes of cryoballoon-based atrial fibrillation ablation. *Am J Cardiol*. 2013;112(4):513–519. doi:10.1016/j.amjcard.2013.04.015. [PubMed: 23672989] *This study showed NLR was predictive of atrial fibrillation recurrence following cryoballoon ablation.
62. Guo X, Zhang S, Yan X, et al. Postablation neutrophil/lymphocyte ratio correlates with arrhythmia recurrence after catheter ablation of lone atrial fibrillation. *Chin Med J (Engl)*. 2014;127(6):1033–1038. [PubMed: 24622430]
63. Li S, Yang F, Jing L, et al. Myeloperoxidase and risk of recurrence of atrial fibrillation after catheter ablation. *J Investig Med*. 2013;61(4):722–727. doi:10.2310/JIM.0b013e3182857fa0.
64. Rudolph V, Andri RP, Rudolph TK, et al. Myeloperoxidase acts as a profibrotic mediator of atrial fibrillation. *Nat Med*. 2010;16(4):470–474. doi:10.1038/nm.2124. [PubMed: 20305660]
65. Paschke S, Weidner AF, Paust T, Marti O, Beil M, Ben-Chetrit E. Technical Advance: Inhibition of neutrophil chemotaxis by colchicine is modulated through viscoelastic properties of subcellular compartments. *J Leukoc Biol*. 2013;94(5):1091–1096. doi:10.1189/jlb.1012510. [PubMed: 23901122]
66. Lennerz C, Barman M, Tantawy M, Sopher M, Whittaker P. Colchicine for primary prevention of atrial fibrillation after open-heart surgery: Systematic review and meta-analysis. *Int J Cardiol*. 2017;249:127–137. doi:10.1016/j.ijcard.2017.08.039. [PubMed: 28918897]
67. Deftereos S, Giannopoulos G, Kossyvakis C, et al. Colchicine for Prevention of Early Atrial Fibrillation Recurrence After Pulmonary Vein Isolation A Randomized Controlled Study. 2012. doi:10.1016/j.jacc.2012.07.031. *This study showed colchicine reduced early atrial fibrillation recurrence following pulmonary vein radiofrequency ablation.
68. Canpolat U, Aytemir K, Yorgun H, et al. The role of preprocedural monocyte-to-high-density lipoprotein ratio in prediction of atrial fibrillation recurrence after cryoballoon-based catheter ablation. *Europace*. 2015;17(12):1807–1815. doi:10.1093/europace/euu291. [PubMed: 25995388]
69. Gunes HM. Relationship between serum osteopontin levels and atrial fibrillation recurrence in patients undergoing cryoballoon catheter ablation. *Turk Kardiyol Dern Arsivi-Archives Turkish Soc Cardiol*. 2016;45(1):26–32. doi:10.5543/tkda.2016.21855.
70. Tian Y, Wang Y, Chen W, Yin Y, Qin M. Role of serum TGF-1 level in atrial fibrosis and outcome after catheter ablation for paroxysmal atrial fibrillation. *Medicine (Baltimore)*. 2017;96(51):e9210. doi:10.1097/MD.00000000000009210. [PubMed: 29390467]
71. Kishima H, Mine T, Takahashi S, Ashida K, Ishihara M, Masuyama T. The Impact of Transforming Growth Factor-1 Level on Outcome After Catheter Ablation in Patients With Atrial Fibrillation. *J Cardiovasc Electrophysiol*. 2017;28(4):402–409. doi:10.1111/jce.13169. [PubMed: 28127812]
72. Wu H, Xie J, Li G, et al. Possible involvement of TGF- / periostin in fibrosis of right atrial appendages in patients with atrial fibrillation. 2015;8(6):6859–6869.
73. 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. doi:10.1007/s10840-013-9867-6. [PubMed: 24554250]
74. Wu CH, Hu YF, Chou CY, et al. Transforming growth factor-1 level and outcome after catheter ablation for nonparoxysmal atrial fibrillation. *Heart Rhythm*. 2013;10(1):10–15. doi:10.1016/j.hrthm.2012.09.016.
75. Wang W, Liu L, Li Y, Hu S, Song Y, Wang X. Does the Expression of Transforming Growth Factor -1. :17–24.
76. Cao H, Zhou Q, Lan R, et al. A functional polymorphism C-509t in TGF-1 promoter contributes to susceptibility and prognosis of lone atrial fibrillation in chinese population. *PLoS One*. 2014;9(11). doi:10.1371/journal.pone.0112912.
77. Wu H, Xie J, Li G-N, et al. Possible involvement of TGF-/periostin in fibrosis of right atrial appendages in patients with atrial fibrillation. *Int J Clin Exp Pathol*. 2015;8(6):6859–6869. www.ijcep.com. Accessed April 13, 2018. [PubMed: 26261573]

78. Verheule S, Sat T, Everett T, IV, et al. Increased vulnerability to atrial fibrillation in transgenic mice with selective atrial fibrosis caused by overexpression of TGF- β 1. *Circ Res*. 2004;94(11):1458–1465. doi:10.1161/01.RES.0000129579.59664.9d. [PubMed: 15117823]
79. Lin CS, Pan CH. Regulatory mechanisms of atrial fibrotic remodeling in atrial fibrillation. *Cell Mol Life Sci*. 2008;65(10):1489–1508. doi:10.1007/s00018-008-7408-8. [PubMed: 18322650]
80. Nattel S, Harada M. Atrial remodeling and atrial fibrillation: Recent advances and translational perspectives. *J Am Coll Cardiol*. 2014;63(22):2335–2345. doi:10.1016/j.jacc.2014.02.555. [PubMed: 24613319]
81. Midgley AC, Rogers M, Hallett MB, et al. Transforming Growth Factor- β 1 (TGF- β 1)-stimulated Fibroblast to Myofibroblast Differentiation Is Mediated by Hyaluronan (HA)-facilitated Epidermal Growth Factor Receptor (EGFR) and CD44 Co-localization in Lipid Rafts *. 2013. doi:10.1074/jbc.M113.451336.
82. Quan TE, Cowper SE, Bucala R. The role of circulating fibrocytes in fibrosis. *Curr Rheumatol Rep*. 2006;8(2):145–150. <http://www.ncbi.nlm.nih.gov/pubmed/16569374>. Accessed May 25, 2018. [PubMed: 16569374]
83. Liu Y, Niu X-H, Yin X, et al. Elevated Circulating Fibrocytes Is a Marker of Left Atrial Fibrosis and Recurrence of Persistent Atrial Fibrillation. *J Am Heart Assoc*. 2018;7(6):e008083. doi:10.1161/JAHA.117.008083. [PubMed: 29535140] **This study found fibrocytes were predictive of atrial fibrillation recurrence in patients undergoing ablation
84. Boldt A, Wetzel U, Lauschke J, et al. Fibrosis in left atrial tissue of patients with atrial fibrillation with and without underlying mitral valve disease. *Heart*. 2004;90(4):400–405. doi:10.1136/HRT.2003.015347. [PubMed: 15020515]
85. Goudis CA, Kallergis EM, Vardas PE. Extracellular matrix alterations in the atria: insights into the mechanisms and perpetuation of atrial fibrillation. *Europace*. 2012;14(5):623–630. doi:10.1093/europace/eur398. [PubMed: 22237583]
86. Kallergis EM, Manios EG, Kanoupakis EM, et al. Extracellular Matrix Alterations in Patients With Paroxysmal and Persistent Atrial Fibrillation. *J Am Coll Cardiol*. 2008;52(3):211–215. doi:10.1016/j.jacc.2008.03.045. [PubMed: 18617070]
87. Swartz MF, Fink GW, Sarwar MF, et al. Elevated pre-operative serum peptides for collagen I and III synthesis result in post-surgical atrial fibrillation. *J Am Coll Cardiol*. 2012;60(18):1799–1806. doi:10.1016/j.jacc.2012.06.048. [PubMed: 23040566]
88. Marchese P, Bursi F, Delle Donne G, et al. Indexed left atrial volume predicts the recurrence of non-valvular atrial fibrillation after successful cardioversion. *Eur J Echocardiogr*. 2011;12(3):214–221. doi:10.1093/ejechocard/jeq176. [PubMed: 21149290]
89. Ejima K, Kato K, Arai K, et al. Impact of atrial remodeling on the outcome of radiofrequency catheter ablation of paroxysmal atrial fibrillation. *Circ J*. 2014;78(4):872–877. <http://www.ncbi.nlm.nih.gov/pubmed/24562637>. Accessed May 3, 2018. [PubMed: 24562637]
90. Grubitzsch H, Grabow C, Orawa H, Konertz W. Factors predicting the time until atrial fibrillation recurrence after concomitant left atrial ablation. *Eur J Cardio-Thoracic Surg*. 2008;34(1):67–72. doi:10.1016/j.ejcts.2008.03.054.
91. Moon J, Lee H-J, Kim J-Y, et al. Prognostic Implications of Right and Left Atrial Enlargement after Radiofrequency Catheter Ablation in Patients with Nonvalvular Atrial Fibrillation. *Korean Circ J*. 2015;45(4):301–309. doi:10.4070/kcj.2015.45.4.301. [PubMed: 26240584]
92. Okumura Y, Watanabe I, Nakai T, et al. Impact of Biomarkers of Inflammation and Extracellular Matrix Turnover on the Outcome of Atrial Fibrillation Ablation: Importance of Matrix Metalloproteinase-2 as a Predictor of Atrial Fibrillation Recurrence. *J Cardiovasc Electrophysiol*. 2011;22(9):987–993. doi:10.1111/j.1540-8167.2011.02059.x. [PubMed: 21489023]
93. Begg GA, Karim R, Oesterlein T, et al. Left atrial voltage, circulating biomarkers of fibrosis, and atrial fibrillation ablation. A prospective cohort study. doi:10.1371/journal.pone.0189936.
94. Boixel C, Fontaine V, Rücker-Martin C, et al. Fibrosis of the left atria during progression of heart failure is associated with increased matrix metalloproteinases in the rat. *J Am Coll Cardiol*. 2003;42(2):336–344. doi:10.1016/S0735-1097(03)00578-3. [PubMed: 12875773]
95. Mukherjee R, Herron AR, Lowry AS, et al. Selective induction of matrix metalloproteinases and tissue inhibitor of metalloproteinases in atrial and ventricular myocardium in patients with atrial

- fibrillation. *Am J Cardiol.* 2006;97(4):532–537. doi:10.1016/j.amjcard.2005.08.073. [PubMed: 16461051]
96. Winkle RA, Jarman JW, Mead RH, et al. Predicting atrial fibrillation ablation outcome: The CAAP-AF score. *Heart Rhythm.* 2016 11;13(11):2119–2125 [PubMed: 27435586]
 97. Quan D, Huang H, Kong B, et al. Predictors of late atrial fibrillation recurrence after cryoballoon-based pulmonary vein isolation: a meta-analysis. *Kardiol Pol.* 2017;75(4):376–385. [PubMed: 28150281]
 98. Hwang HJ, Son JW, Nam BH, et al. Incremental predictive value of pre-procedural N-terminal pro-B-type natriuretic peptide for short-term recurrence in atrial fibrillation ablation. *Clin Res Cardiol.* 2009 4;98(4):213–8. [PubMed: 19229464]
 99. Solheim E, Off MK, Hoff PI, et al. N-terminal pro-B-type natriuretic peptide level at long-term follow-up after atrial fibrillation ablation: a marker of reverse atrial remodelling and successful ablation. *J Interv Card Electrophysiol.* 2012 8;34(2):129–36. [PubMed: 21993599]
 100. Hussein AA, Saliba WI, Barakat A, et al. Radiofrequency Ablation of Persistent Atrial Fibrillation: Diagnosis-to-Ablation Time, Markers of Pathways of Atrial Remodeling, and Outcomes. *Circ Arrhythm Electrophysiol.* 2016 1;9(1):e003669. [PubMed: 26763227]
 101. Richter B, Gwechenberger M, Socas A, et al. Time course of markers of tissue repair after ablation of atrial fibrillation and their relation to left atrial structural changes and clinical ablation outcome. *Int J Cardiol.* 2011 10 20;152(2):231–6. [PubMed: 20692054]
 102. On YK, Jeon ES, Lee SY, et al. Plasma transforming growth factor beta1 as a biochemical marker to predict the persistence of atrial fibrillation after the surgical maze procedure. *J Thorac Cardiovasc Surg.* 2009 6;137(6):1515–20. [PubMed: 19464473]
 103. Gaynor SL, Schuessler RB, Bailey MS, et al. Surgical treatment of atrial fibrillation: predictors of late recurrence. *J Thorac Cardiovasc Surg.* 2005 1;129(1):104–11. [PubMed: 15632831]
 104. Genev IK, Tompkins LA, Khare MM, Farokhi F. Comparison of the Efficacy and Complication Rates of the Hybrid Maze, Complete Cox-Maze and Catheter Ablation in the Treatment of Atrial Fibrillation. *J Atr Fibrillation.* 2017 2 28;9(5):1543. [PubMed: 29250276]
 105. Bogachev-Prokophiev A, Zheleznev S, Pivkin A, et al. Assessment of concomitant paroxysmal atrial fibrillation ablation in mitral valve surgery patients based on continuous monitoring: does a different lesion set matter? *Interact Cardiovasc Thorac Surg.* 2014 2;18(2):177–81. [PubMed: 24254537]
 106. Phan K, Xie A, Kumar N, et al. Comparing energy sources for surgical ablation of atrial fibrillation: a Bayesian network meta-analysis of randomized, controlled trials. *Eur J Cardiothorac Surg.* 2015 8;48(2):201–11. [PubMed: 25391388]
 107. Kühne M, Suter Y, Altmann D, et al. Cryoballoon versus radiofrequency catheter ablation of paroxysmal atrial fibrillation: biomarkers of myocardial injury, recurrence rates, and pulmonary vein reconnection patterns. *Heart Rhythm.* 2010 12;7(12):1770–6. [PubMed: 20817019]
 108. Jeong DS, You JH, Jeon CS, et al. Impact of ablation duration on rhythm outcome after concomitant maze procedure using cryoablation in patients with persistent atrial fibrillation. *J Cardiothorac Surg.* 2017 7 24;12(1):60. [PubMed: 28738900]
 109. Chang S, Chen Y, Hsu C, et al. Heat shock protein inducer modifies arrhythmogenic substrate and inhibits atrial fibrillation in the failing heart. *Int J Cardiol.* 2013;168(4):4019–4026. doi: 10.1016/j.ijcard.2013.06.072. [PubMed: 23871620]
 110. Lanters EAH, van Marion DMS, Kik C, et al. HALT & REVERSE: Hsf1 activators lower cardiomyocyte damage; towards a novel approach to REVERSE atrial fibrillation. *J Transl Med.* 2015;13:347. doi:10.1186/s12967-015-0714-7. [PubMed: 26541406]
 111. Musumeci D, Roviello GN, Montesarchio D. An overview on HMGB1 inhibitors as potential therapeutic agents in HMGB1-related pathologies. *Pharmacol Ther.* 2014;141(3):347–357. doi: 10.1016/j.pharmthera.2013.11.001. [PubMed: 24220159]
 112. Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1 β inhibition and the prevention of recurrent cardiovascular events: Rationale and Design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). *Am Heart J.* 2011;162(4):597–605. doi:10.1016/j.ahj.2011.06.012. [PubMed: 21982649]

113. Bang CN, Greve AM, Abdulla J, Køber L, Gislason GH, Wachtell K. The preventive effect of statin therapy on new-onset and recurrent atrial fibrillation in patients not undergoing invasive cardiac interventions. *Int J Cardiol.* 2013;167(3):624–630. doi:10.1016/j.ijcard.2012.08.056. [PubMed: 22999824]
114. Zhang X-D, Gu J, Jiang W-F, et al. Optimal rhythm-control strategy for recurrent atrial tachycardia after catheter ablation of persistent atrial fibrillation: a randomized clinical trial. *Eur Heart J.* 2014;35(20):1327–1334. doi:10.1093/eurheartj/ehu017. [PubMed: 24497338]
115. Barekattain A, Razavi M. Antiarrhythmic therapy in atrial fibrillation: indications, guidelines, and safety. *Tex Heart Inst J.* 2012;39(4):532–4. [PubMed: 22949771]
116. Singla S, Karam P, Deshmukh AJ, Mehta J, Paydak H. Review of contemporary antiarrhythmic drug therapy for maintenance of sinus rhythm in atrial fibrillation. *J Cardiovasc Pharmacol Ther.* 2012;17(1):12–20. [PubMed: 21335483]
117. Xu B, Peng F, Tang W, Du Y, Guo H. Short-term Antiarrhythmic Drugs After Catheter Ablation for Atrial Fibrillation: A Meta-analysis of Randomized Controlled Trials. *Ann Pharmacother.* 2016;50(9):697–705. [PubMed: 27317015]
118. Chen W, Liu H, Ling Z, et al. Efficacy of Short-Term Antiarrhythmic Drugs Use after Catheter Ablation of Atrial Fibrillation-A Systematic Review with Meta-Analyses and Trial Sequential Analyses of Randomized Controlled Trials. *PLoS One.* 2016;11(5):e0156121. [PubMed: 27224469]
119. Ad N, Holmes SD, Shuman DJ, Pritchard G, Miller CE. Amiodarone after surgical ablation for atrial fibrillation: Is it really necessary? A prospective randomized controlled trial. *J Thorac Cardiovasc Surg.* 2016;151(3):798–803. [PubMed: 26253874]

11.

Key issues

- DAMPs such as HMGB-1, TLRs, RAGE, and the NLRP3 inflammasome may be associated with pathogenesis of AF and RAF, but due to their ubiquitous nature are unlikely to be specific biomarkers.
- HSPs may serve as biomarkers to predict RAF following ablation therapy. The differing effects associated with intracellular and extracellular HSPs, along with anti-HSP antibodies warrant further investigation. HSP27 shows the most promise as a biomarker.
- Inflammatory cytokines such as: CRP, IL-2, IL-6, and TNF- α have not been shown to be reliable predictors of RAF. Inflammation has been associated with RAF, but recent studies suggest it may be a consequence of AF rather than a cause.
- Collagen deposition and metabolism show promise as predictive biomarkers of RAF. TGF- β shows the most promise as a cytokine to be used as a biomarker. More studies investigating collagen deposition and breakdown are warranted.
- Several ratios evaluating the activity of inflammatory cells may show promise to predict RAF. Currently, the activities of PMNs and monocytes have been evaluated, but recent studies suggest fibrocytes may play a role in atrial remodeling.

Figure 1:

This figure depicts the proposed immunopathogenesis of recurrent atrial fibrillation in the setting of preexisting atrial fibrillation. Existing atrial fibrillation is often due to ectopic focal firing, reentrant pathways, heterogenous impulse conduction, atrial remodeling, and is often associated with background inflammation. Atrial fibrillation is often treated via ablation therapy. Ablation therapy may result in increased levels of damage-associated molecular patterns (DAMPs), inflammatory cytokines, inflammatory cell migration and activity, and collagen remodeling. Together these processes are believed to contribute to atrial remodeling and ultimately recurrent atrial fibrillation. The second half of the diagram below the dashed line shows more specific mediators believed to play a role in the immunopathogenesis of recurrent atrial fibrillation. DAMPs, such as high mobility group box-1 (HMGB-1), mitochondrial DNA (mtDNA), and NLRP3 inflammasome, may be released from cardiomyocytes in response to stress and generate a sterile immune response causing the release of inflammatory cytokines through toll-like receptor (TLR) activation. DAMPs, including heat shock proteins (HSP) 27, 70, and 60, may also play a role in the sterile immune response associated with increased DAMP levels. However, certain HSPs may also have a cardioprotective effect and do not contribute to the pathogenesis of recurrent atrial fibrillation. Inflammatory cytokines, such as C-reactive protein (CRP), tumor necrosis factor-alpha (TNF- α), interleukin-2 (IL-2), and interleukin-6 (IL-6), have been associated with recurrent atrial fibrillation. The major contribution of these inflammatory

cytokines in the progression of recurrent atrial fibrillation includes the recruitment of inflammatory cells such as polymorphonucleocytes (PMNs), macrophages, and fibroblasts. Once recruited, these cells infiltrate the atria and begin removing cell debris and increase metabolism of the extracellular matrix (ECM). Additionally, cytokines such as transforming growth factor-beta 1 (TGF- β 1) stimulate fibroblasts to increase ECM, mainly through collagen deposition. TGF- β 1 may be secreted in response to increased levels of angiotensin-II (AT-II). Increased inflammatory cell activity leads to increased ECM metabolism and ultimately fibrotic remodeling of the atria, which is believed to be the major factor leading to recurrent atrial fibrillation.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2:

This figure depicts different roles of intracellular and extracellular heat shock proteins (HSPs). The left panel demonstrates the protective effects of intracellular HSPs. Cells are exposed to stress such as ischemia, reactive oxygen species, or inflammation, which result in increased activity of intracellular HSPs like HSP27, HSP70 and HSP60. These HSPs act as molecular chaperones to prevent protein misfolding and degradation, and ultimately serve to mitigate cellular remodeling. The panel on the right depicts the events when a cardiomyocyte is exposed to severe stress. The cardiomyocyte responds by upregulating HSPs until they are present on the surface of the cell in the extracellular environment. Extracellular HSPs are proinflammatory, and their presence may result in myocyte injury or myolysis, contributing to atrial remodeling.

Figure 3:

This figure depicts the potential mechanism of collagen deposition associated with atrial fibrosis. TGF-β1 transforms fibroblast into myofibroblast and encourages procollagen synthesis. Inflammatory cytokines, including IL-2, IL-6, TNF-α, act with myofibroblasts to promote collagen deposition. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) influence ECM remodeling and create collagen breakdown

byproducts. Fibrotic remodeling is the strongest factor perpetuating atrial fibrillation and is most likely the most influential process driving recurrence.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript