

HIGH SENSITIVITY C-REACTIVE PROTEIN AND RISK OF STROKE IN ATRIAL  
FIBRILLATION: THE REASONS FOR GEOGRAPHIC AND RACIAL DIFFERENCES IN  
STROKE (REGARDS) STUDY

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## LIST OF ABBREVIATIONS

AF= Atrial Fibrillation

CHADS2 score = congestive heart failure, hypertension, age >75, diabetes, stroke or

Transient ischemic attack

DM = Diabetes Mellitus

ECG= Electrocardiogram

EPI CARE= Epidemiological Cardiology Research Center

HR= Hazard Ratio

Hs-CRP= high sensitivity- C reactive protein

HTN= Hypertension

REGARDS= Reasons for Geographic and Racial Differences in Stroke

SD= Standard Deviation

TIA= Transient Ischemic Attack

## ABSTARCT

**Background and Purpose-** High sensitivity C-reactive protein (hs-CRP), a marker of systemic inflammation, is a predictor of cardiovascular events in the general population. We compared the association between hs-CRP level and subsequent stroke events in individuals with and without atrial fibrillation (AF) in the REGARDS study.

**Methods –** A total of 27, 609 participants (40% African American, 54% female, age $\geq$  45 years) of whom 2,408 had AF were included in this analysis. Stroke cases were identified and adjudicated during 8.3 years follow up (median 5 years). AF was ascertained by electrocardiogram or self- reported history of previous physician diagnosis. Cox proportional hazard analysis was used to examine the risk of stroke in study participants with and without AF, separately.

**Results-** Median hs-CRP was 2.7 mg/L (IQR: 5.0) in the AF group and 2.2 mg/L (IQR: 4.0) in the group without AF (p log hs-CRP <0.01). Stroke free survival curves in AF population with hs-CRP less vs greater than 3mg/L was not statistically significant (p 0.78) but appeared significant in those without AF (p<0.01). In a model adjusted for demographics, traditional stroke risk factors, and warfarin use, one unit change in hs-CRP was associated with subsequent stroke in the participants without AF (HR 1.01 95%CI 1.01, 1.02) but not in those with AF (HR 1.00 (95% CI 0.97-1.02)).

**Conclusions-** In the REGARDS study, hs-CRP was significantly associated with stroke risk in population without AF but not in those with AF. These findings suggest a limited value of hs-CRP in improving stroke risk stratification in patients with AF.

**Keywords:** AF, ischemic stroke, inflammation

## CHAPTETR 1

### BACKGROUND

#### I. C- Reactive Protein (CRP)

C-reactive protein was discovered in the laboratory of Oswald T. Avery while clinical studies were underway to develop remedies for pneumococcal pneumonia. It was in 1930 when William S. Tillett and Thomas Francis, Jr. discovered a new antigen of the bacteria pneumococcus which was called Fraction C. <sup>1</sup>Tillett and Francis noted that it caused a strong precipitation reaction in the early phase of infection which diminished and eventually disappeared as the patients recovered. Several years later, Avery, Theodore J. Abernethy and Colin MacLeod established the so-called C-reactive substance was a protein and described many of its properties.

The protein belongs to the pentraxin family (from the Greek penta (five) and ragos (barries) based on appearance under electron microscopy) of calcium dependent ligand-binding plasma proteins. The dynamic protein is synthesized primarily by hepatocytes and under the transcriptional control of the cytokine Interleukin-6 (IL-6).<sup>2</sup> It is considered an acute phase reactant since plasma concentration increases by at least 25% during inflammatory disorders. In the presence of infection, ischemia or tissue damage, the level of this acute phase reactant can increase 10,000 fold.<sup>3</sup> Indeed, de novo hepatic synthesis starts soon after stimulus and its synthesis rate is strictly related to the intensity of pathological event.<sup>4</sup> In the absence of such enticing events, self-correlation coefficient of CRP repeated years apart is about 0.5 which is similar to cholesterol levels and systolic blood pressure.<sup>5</sup> There is no significant seasonal or diurnal variation and it is not affected by eating.<sup>2</sup> The plasma half-life of CRP is about 19 hours and it is constant under all conditions of health and disease. Immunoassays

of CRP with greater sensitivity than those previously used emerged in mid 1990s and the high sensitivity CRP (hs-CRP) replaced previously used assays. In healthy young adult volunteer blood donors, the median concentration of hs-CRP is 0.8 mg/l with the 90<sup>th</sup> percentile being 3mg/l and 99<sup>th</sup> percentile is 10mg/l.<sup>6</sup>

CRP level tends to increase with age presumably correlating with increasing incidence of subclinical disease.<sup>7</sup> There is wealth of evidence in the literature showing ethnic and gender variation in CRP levels. In the National Health and Nutrition Examination Survey (NHANES), a representative sample of US women had higher level of hs-CRP compared to men regardless of hormone replacement therapy use.<sup>8</sup> Despite accounting for common confounding variables, the gender differences in hs-CRP was maintained across multiplet ethnic subgroups (Whites, Blacks, Hispanics and Asians) in the Multiethnic Study of Atherosclerosis (MESA).<sup>9</sup> In the Women's Health Study, similar results were found where median hs-CRP level was significantly higher among black women (2.96 mg/L) than among white (2.02 mg/L), Hispanic (2.06 mg/L), and Asian (1.12 mg/L) counterparts.<sup>10</sup> In The Reasons for Geographically and Racial Differences in Stroke (REGARDS), which is a large study of geographically dispersed white and black men and women, increased hs-CRP was more common among women than men and blacks than white.<sup>11</sup>

## II. Hs-CRP and Cardiovascular Disease (CVD)

Few at the time of discovery of CRP predicated that it would emerge as a powerful surrogate marker of atherothrombosis. In the mid-1990s, studies linking inflammation, using CRP as a surrogate marker, with atherosclerosis began to surface in the US after clinical use of CRP has been largely ignored for thirty years.<sup>12-13</sup> Analysis of CRP values from large epidemiological studies of stored patient sera largely followed due to its

exceptional stability overtime. These studies drew attention to the predictive values of CRP of future CVD events like acute myocardial ischemia, recurrent ischemia and stroke in the general population.<sup>14-19</sup> In 2003, the Center for Disease Control (CDC) and American Heart Association (AHA) recommended measuring hs-CRP in patients with intermediate CVD risk (eg, 10% to 20% risk over 10 years), in whom the physician may need additional information to guide considerations of further evaluation (eg, imaging, exercise testing) or therapy (eg, drug therapies with lipid-lowering, antiplatelet, or cardioprotective agents).<sup>13</sup>

### III. Inflammation and Atrial Fibrillation (AF)

Atrial fibrillation (figure 1) is the most common sustained arrhythmia encountered in clinical practice affecting over two million Americans.<sup>20</sup> This number is expected to increase dramatically to between 6 and 12 million by the year 2050.<sup>21</sup> As the number of patients afflicted with this arrhythmia increase, associated morbidity and mortality will increase, particularly for stroke, as consequence of systemic embolization from the fibrillating left atrium and its appendage.<sup>22-24</sup> Due to the predominance of AF in the elderly and improved survival of patients with CVD, hospitalization for AF has increased by two to three folds.<sup>25-26</sup>

Inflammatory conditions and processes like advanced age, heart failure, hypertension, diabetes mellitus, obesity and cigarette smoking are well known risks for developing AF.<sup>27-30</sup> Evidence for inflammatory contribution to AF is further suggested by high incidence of AF after cardiac surgery (25-40%) which is a known trigger of strong inflammatory response through ischemia, cardiopulmonary bypass and cardiomy.<sup>31</sup> Bruins et al<sup>32</sup> reported that an IL-6 level, which is a cytokine responsible for stimulating CRP production, rise markedly after surgery and peak at 6 hours. A

second phase then occurs with an increase in CRP, which peaks on the second postoperative day followed by increases in complement-CRP complexes peaking on the second or third postoperative day. The incidence of atrial arrhythmias in fact similarly peaks 2 to 3 days after surgery.

The first observation linking inflammation to atrial fibrillation at tissue level was made by Frustaci et al.<sup>33</sup> who demonstrated a high prevalence of inflammatory infiltrates, myocyte necrosis, and fibrosis in atrial biopsies from 12 patients with lone AF compared to biopsies from control patients. Similar findings were reported by Nakamura et al.<sup>34</sup> in the endothelium of left atrial appendages obtained from seven patients with nonvalvular AF and confirmed in an animal model where dogs with sustained AF demonstrated active atrial perimyocarditis with inflammatory infiltrates, lipid degeneration, and fibrosis.<sup>35</sup>

Two studies that appeared almost simultaneously in the literature were the first to report an association between CRP and AF in non-post-operative patients. The first study was case-control study of CRP in 131 patients with non-postoperative atrial arrhythmias compared with 71 control patients found that CRP is elevated in AF patients and in fact the levels were higher with higher AF burden<sup>36</sup>. The second study compared 50 patients with paroxysmal AF (PAF) with age and sex controls with similar risk factors and found that CRP was significantly higher in patients with PAF and was associated with successful cardioversion back to normal sinus rhythm<sup>37</sup>. The link between CRP and incident AF was later shown in multiple large epidemiological population studies.<sup>38-40</sup>

#### IV. Inflammation and thromboembolism in AF

Several clinical scenarios confirm the relationship between inflammation and thrombus formation like deep vein thrombosis (DVT), sepsis and disseminated intravascular coagulation (DIC). Interestingly, previous studies implicating inflammation as an important feature of thrombosis and also pathogenesis of atrial fibrillation led to speculation that inflammation may also contribute to a pro-thrombotic environment in atrial fibrillation thus facilitating thrombus formation and subsequent embolic complications. Indeed, thrombus formation in AF can be explained by referencing the Virchows triad (figure 2) described 150 years ago. We know that AF results in abnormal flow with resultant irregularly irregular cardiac output and endothelial cardiac tissue damage leading to abnormalities in indices of inflammation like CRP.<sup>41-42</sup> Therefore, the triad of abnormal blood flow, abnormal vessel wall and abnormal blood components is fulfilled.

Left atrial thrombosis causing stroke by arterial embolism has been thought to be the main pathogenetic mechanism in the association between AF and stroke. The incidence of left atrial thrombosis in patients with nonvalvular AF is approximately 10%.<sup>43-44</sup> Furthermore, CRP has been shown to be associated with spontaneous echo contrast in the left atrial appendage which is known independent predictor of stroke in AF.<sup>42</sup> The cytokine IL-6, which controls transcription of CRP, was an independent predictor of vascular events and stroke in 77 patients with AF that were followed for 6.3 years.<sup>45</sup> In a cross sectional study of 191 patients with AF, IL-6 was also correlated with a point-based score for stroke risk in AF.<sup>46</sup> Another cross sectional study of 106 patients with AF and 41 healthy controls found elevated levels of CRP, IL-6 and higher plasma viscosity in AF patients.<sup>42</sup>

Despite the fact that the precise pathophysiologic process underlying the prothrombotic state in AF remains unclear, the above studies point to the important relationship between inflammation in AF and prothrombotic state leading to stroke.

#### V. Atrial Fibrillation and the Risk of Stroke

Stroke is the most dreaded complication of AF and the fifth leading cause of death and disability in the United States. AF prevalence is projected to reach epidemic proportions in coming decades and was shown to be associated with 5-fold increase in the rate of ischemic stroke independent of other cardiovascular contributors.<sup>22</sup> Atrial fibrillation accounts for about 15% of all strokes in the U.S. In fact, the arrhythmia is responsible for about 33% of strokes in persons over 65 years of age. The annual cost burden of stroke due to AF is at least \$9.8 billion per year. According to the AHA statistics, the combined direct and indirect cost of stroke in the US was estimated to be \$65.5 billion in 2008. The majority of costs were due to high hospitalization rates, the need for rehabilitation and long-term care. Thus, the management of atrial fibrillation complications is projected to become an increasingly dominant contributor to health care expenditures in this country.

The incidence of strokes in patients with nonvalvular AF reported in several control studies varied from 5.5% in the Copenhagen AFASAK study, 6.3% in the Stroke Prevention in Atrial Fibrillation (SPAF) study, 3.0% in the Boston Area Anticoagulation Trial for Atrial Fibrillation (BAATAF) study, 5.2% in the Canadian Atrial Fibrillation Anticoagulation (CAFA) study and 4.3% in the Veterans Affairs Stroke Prevention in Nonrheumatic Atrial Fibrillation (SPINAF) study.<sup>47-51</sup> The risk of stroke attributed to AF rises from 1.5% for persons age 50-59 to 23.5% for those aged 80-89 which highlights that the elderly represents the vulnerable population.<sup>22</sup> In fact, patients who present

with stroke due to AF have worse outcome in terms of morbidity, mortality and length of hospital stay, compared to stroke in the absence of AF.<sup>25-26</sup> In addition, the recurrence of stroke in AF may be as high as 12% per year and more than 50% of survivors remain with a severe deficit.<sup>52</sup>

Five randomized trials aimed to investigate whether anticoagulation with warfarin would reduce risk of stroke and formulating risk-stratification schemes for stroke prevention in patients with nonvalvular AF: The Boston Area Anticoagulation Trial for Atrial Fibrillation (BAATAF)<sup>51</sup>, Stroke Prevention in Atrial Fibrillation Study (SPAF)<sup>50</sup>, Veterans Affairs Stroke Prevention in Nonrheumatic Atrial Fibrillation (Veteran Affairs SPINAF)<sup>53</sup>, The Copenhagen AFASAK study (AFASAK)<sup>49</sup>, Canadian Atrial Fibrillation Anticoagulation Study (CAFA)<sup>52</sup>. However, the endpoint event rates in the control groups of each trial did not permit risk stratification with a high degree of confidence. Atrial Fibrillation Investigators (AFI) pooled the data from these five prevention trials in an attempt to develop a stronger risk-stratification scheme.<sup>53</sup> Four independent risk factors for embolism in AF were identified: Age greater than 65 years, history of stroke or transient ischemic attack (TIA), diabetes mellitus (DM) and history of systemic hypertension (HTN). Patients with AF and none of the risk factors were found to be a low-risk with an annual stroke risk of ~ 1% without anticoagulation while patients with at least one risk factor had stroke rates of at least 4% per year. The risk stratification scheme derived from the Stroke Prevention in Atrial Fibrillation III Study (SPAF III)<sup>54-55</sup> of nonvalvular AF, which included patient with low risk of stroke treated with aspirin and high risk patients treated with dose adjusted warfarin with and without aspirin, identified the following clinical risk factors for embolism in AF leading to stroke: women greater than 75 years, history of stroke or TIA, impaired LV systolic function (clinical heart

failure [HF] in the last three months or fractional shortening less than 25% on transthoracic echocardiography, systolic blood pressure  $\geq 160$  mmHg. The CHADS2 score, which was derived from the AFI and SPAF models, is the most widely used clinical model for assessment of stroke risk for patients with nonvalvular AF:

- Congestive heart failure (HF, any history) (1 point)
- Hypertension (prior history) (1 point)
- Age  $\geq 75$  years (1 point)
- Diabetes mellitus (1 point)
- Secondary prevention in patients with ischemic stroke or TIA (2 point2)

#### VI. Rational and Research Question

Baseline hs-CRP, an acute phase reactant and an indicator of systemic inflammation, has been shown to be consistently associated with atherothrombotic disease in epidemiological studies of patients with acute myocardial ischemia, recurrent ischemia and stroke.<sup>56-61</sup> Few of these studies were large prospective studies that used stroke as primary outcome and have primarily focused on selected population of men, women and whites and even fewer on those with atrial fibrillation.<sup>42, 62-63</sup> Despite the considerable evidence demonstrating that AF is intimately associated with an inflammatory state, most of the studies are limited to animal models, post-operative cardiac patients or small number of participants in observational studies.

Previous studies have demonstrated increased risk of thrombogenesis in patients with AF suggesting the presence of a hypercoagulable or prothrombotic state leading to increased risk of stroke yet the exact mechanism continues to be unclear. Increasing evidence suggests that the thrombogenic tendency in AF may be related to abnormal changes in inflammatory blood constituents like CRP. Better understanding of the

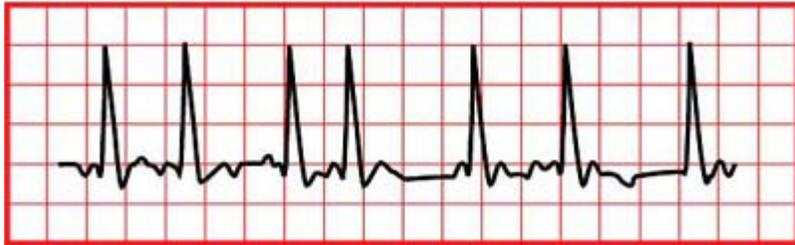
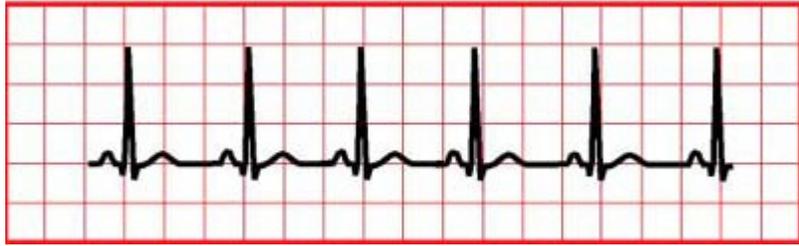
pathophysiology of inflammation in AF which contributes to the risk of stroke can change clinical management and complement current stroke risk stratification schema.

To further investigate the apparent link between thrombogenesis and inflammation in AF, we hypothesize that systemic inflammation as indicated by hs-CRP level would be associated with ischemic stroke especially in AF patients with baseline hs-CRP level greater than 3 mg/l which has been known to be a risk factor for atherothrombotic disease. To test this hypothesis, we will compare hs-CRP measured at baseline in the REasons for Geographic And Racial Differences in Stroke (REGARDS) study participants with AF as it relates to subsequent stroke. The study will help fill in the knowledge gap in terms of the utility of hs-CR as clinical screening marker to identify patients with increased risk of stroke in the setting of known AF and further clarify the relationship between inflammation, AF and ischemic stroke. If an association is found, it will allow us to refine current stroke risk stratification schema, like the CHADS<sub>2</sub>, to better identify patients with increased risk for stroke events and may potentially lead to novel therapeutic interventions to reduce the burden of AF and associated strokes.

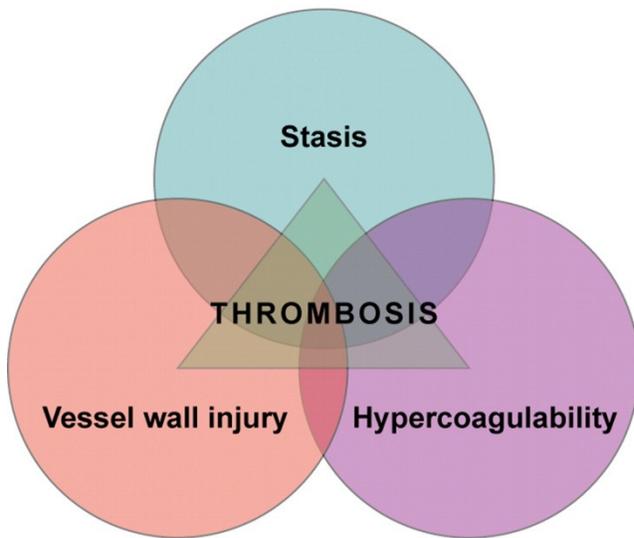
## VII. Specific Aims

- ❑ Primary aim: Examine whether baseline hs-CRP  $> 3\text{mg/l}$  compared to hs- CRP level  $\leq 3\text{ mg/l}$  is associated with increased risk of subsequent stroke in REGARDS participants with AF.
- ❑ Secondary aim: Examine whether baseline hs-CRP  $> 3\text{mg/l}$  compared to hs- CRP level  $\leq 3\text{ mg/l}$  is associated with increased risk of subsequent stroke in REGARDS participants without AF.

**Figure 1.** A) ECG showing normal sinus rhythm, B) ECG showing atrial fibrillation



**Figure 2.** Virchow's triad



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## CHAPTER 2

### HIGH SENSITIVITY C-REACTIVE PROTEIN AND RISK OF STROKE IN ATRIAL FIBRILLATION: THE REASONS FOR GEOGRAPHIC AND RACIAL DIFFERENCES IN STROKE (REGARDS) STUDY

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## **Abstract**

**Background and Purpose-** High sensitivity C-reactive protein (hs-CRP), a marker of systemic inflammation, is a predictor of cardiovascular events in the general population. We compared the association between hs-CRP level and subsequent stroke events in individuals with and without atrial fibrillation (AF) in the REGARDS study.

**Methods** – A total of 27, 609 participants (40% African American, 54% female, age > 45 years) of whom 2,408 had AF were included in this analysis. Stroke cases were identified and adjudicated during 8.3 years follow up (median 5 years). AF was ascertained by electrocardiogram or self-reported history of previous physician diagnosis. Cox proportional hazard analysis was used to examine the risk of stroke in study participants with and without AF, separately.

**Results-** Median hs-CRP was 2.7 mg/L (IQR: 5.0) in the AF group and 2.2 mg/L (IQR: 4.0) in the group without AF ( $p$  log hs-CRP <0.01). Stroke free survival curves in AF population with hs-CRP less vs greater than 3mg/L was not statistically significant ( $p$  0.78) but appeared significant in those without AF ( $p$ <0.01). In a model adjusted for demographics, traditional stroke risk factors, and warfarin use, one unit change in hs-CRP was associated with subsequent stroke in the participants without AF (HR 1.01 95%CI 1.01, 1.02) but not in those with AF (HR 1.00 (95% CI 0.97-1.02)).

**Conclusions-** In the REGARDS study, hs-CRP was significantly associated with stroke risk in population without AF but not in those with AF. These findings suggest a limited value of hs-CRP in improving stroke risk stratification in patients with AF.

**Keywords:** AF, ischemic stroke, inflammation

## Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia encountered in clinical practice affecting over 2 million Americans and this number is expected to reach 6-12 million by 2050.<sup>1,2</sup> It is associated with adverse prognosis being an independent risk factor for stroke, myocardial infarction and death.<sup>3-5</sup> Furthermore, patients who present with stroke due to AF have worse outcomes including morbidity, length of hospital stay and mortality compared to stroke in the absence of AF<sup>6,7</sup>.

Previous studies have demonstrated increased risk of thrombogenesis in patients with AF suggesting the presence of a hypercoagulable or prothrombotic state leading to increased risk of stroke. The intimate relationship between inflammation and prothrombotic state in AF is well recognized.<sup>8</sup> Increasing evidence suggests that the thrombogenic tendency in AF may be related to abnormal changes in inflammatory blood constituents like CRP.<sup>9</sup> Furthermore, Inflammatory conditions like advanced age, heart failure, hypertension, diabetes mellitus, obesity and cigarette smoking are well known risks for developing AF.<sup>10</sup>

High sensitivity CRP, an acute phase reactant and an indicator of systemic inflammation, has been shown to be consistently associated with atherothrombotic disease in patients with acute myocardial ischemia, recurrent ischemia and stroke.<sup>11-16</sup> Few of these studies were large prospective studies, used stroke as primary outcome, included an ethnically diverse population<sup>17-22</sup> and even fewer focused on individuals with AF.<sup>23-24</sup> To address the issue of inflammation as a risk for stroke in AF compared to the general population, we examined and compared the relationship between baseline hs-CRP level and stroke events in participants with and without AF in the

REGARDS cohort. This would shed light on the potential utility of hs-CRP as a screening tool, independently or as an added component to the current risk stratification scores to identify patients with increased risk of thromboembolic event in the setting of known AF.

## **Methods**

### **Study population**

The goals and design of the REGARDS study were published elsewhere.<sup>25</sup> Briefly, the study was designed to investigate the causes of regional and racial disparities in stroke mortality, oversampling blacks and residents of the southeastern stroke belt region (North Carolina, South Carolina, Georgia, Alabama, Mississippi, Tennessee, Arkansas, and Louisiana). Individuals were recruited from a commercially available list of residents using a combination of postal and telephone contact with a 49% cooperation rate. Using a computer-assisted telephone interview, trained interviewers obtained demographic information and a cardiovascular medical history. Consent was obtained initially on the telephone and subsequently in writing during an in-person evaluation. In-home brief physical examinations were conducted 3 to 4 weeks after the telephone interview. Participants are followed every 6 months by telephone for possible stroke outcomes. Of the 30,183 REGARDS participants enrolled between 2003 and October 2007, we excluded 702 with missing AF data and 1,872 with no hs-CRP level recorded, resulting in 27,609 (91.5%) participants for analysis. Stroke was defined as first time and recurrent stroke and included ischemic and hemorrhagic stroke.

## **AF Diagnosis**

Information on AF ascertainment was previously published<sup>26,27</sup>. AF was diagnosed based on baseline ECG and self-reported history of a previous physician diagnosis. Baseline ECG was recorded during the in-home visits by a healthcare professional. Staff members were trained on standard procedures with use of centrally trained supervisors, a web-based program, and continuous quality feedback to individual examiners. The ECGs were sent to a central ECG reading center (EPICARE Center, Wake Forest School of Medicine, Winston-Salem, NC) where they were read, coded, and interpreted by electrocardiographers blinded to clinical data. Self-reported previous physician diagnosis of AF was defined as a positive response to the question, “Has a physician or a health professional ever told you that you had AF?”

## **Stroke Events**

Details on stroke events identification and adjudication have been previously published.<sup>28</sup> In summary, report of a possible stroke/transient ischemic attack or a positive response to the stroke symptoms on the Questionnaire for Verifying Stroke-Free Status<sup>29</sup>, resulting in hospitalization and follow-up generated a request for retrieval of medical records that were centrally adjudicated by a panel of stroke expert physicians. Stroke events were defined according to the following World Health Organization definition as “rapidly developing clinical signs of focal, at times global, disturbance of cerebral function, lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin”.<sup>30</sup> Events not meeting the World Health Organization definition but characterized by symptoms lasting less than 24 h with neuroimaging consistent with acute ischemia or hemorrhage were classified as clinical

strokes. This analysis included World Health Organization (WHO) defined and clinical ischemic stroke cases.

### **Covariates**

Standardized physical measures including height, weight and blood pressure were collected at the in-home physical examination. Demographics (age, sex, and race) were defined by self-report. Stroke risk factors were selected based on the components of the CHADS2 Risk Score.<sup>31</sup> Congestive heart failure was defined as self-report of both orthopnea, defined as answering positively to “Do you ever have to sleep on  $\geq 2$  pillows to help you breathe?” and paroxysmal nocturnal dyspnea, defined as answering positively to “Do you ever wake at night because you are having trouble breathing?” Hypertension was defined as at least 1 of the following: systolic blood pressure  $\geq 140$  mm Hg, diastolic blood pressure  $\geq 90$  mm Hg or a self-report of currently taking medication to control blood pressure. Diabetes mellitus was defined as at least 1 of the following: fasting blood glucose  $\geq 126$  mg/dl., nonfasting glucose  $\geq 200$  mg/dl, or a self-report of currently taking an oral hypoglycemic agent or insulin.

Current smoking and previous CVD (defined by self-report (myocardial infarction or heart attack, coronary artery bypass surgery, coronary angioplasty, or stenting) or by ECG evidence of a previous myocardial infarction) were included. Use of antihypertensive, lipid lowering, and antihyperglycemic medications, current warfarin treatment and consistent aspirin use were defined using an inventory of current medications that was conducted during the in-home visits.

## **Statistical Analysis**

Frequency distributions of all variables were first inspected to identify anomalies and outliers possibly caused by measurement artifacts. Continuous data were described by their mean and SD and categorical data as proportions (percentage). Stroke cases were defined as new and recurrent stroke during follow up. Participants without incident or recurrent stroke were censored at death or last follow-up.

Cox proportional hazards analysis was used to examine the hazard ratios (HR) and 95% confidence interval (CI) for the association of stroke with hs-CRP per one unit change in hs-CRP in the participants with AF (self-reported and ECG-detected AF collectively) compared to participants without AF, separately. Models created were first unadjusted (Model 1), adjusted for baseline demographics like age, sex, and race (Model 2), and then further adjusted for components of the CHADS2 score( congestive heart failure, hypertension, diabetes and previous stroke or TIA) along with warfarin and/or aspirin use (Model 3). Analyses are reported using one unit change in hs-CRP and cut point of 3 mg/dl, which was shown to be associated with increased CVD risk. Analyses were repeated using median specific hs-CRP cut points stratified by sex and race and hs- CRP quartile cut points without change in qualitative results. Stroke free survival curves in AF population with hs-CRP less vs greater than 3mg/L were also created.

## **Results**

A total of 27, 609 participants (40% African American, 54% female, age > 45 years) included in this analysis. AF was detected in 2084 of participants. At baseline, hs-CRP was not normally distributed with median of 2.7 mg/L (IQR: 5.0) in the AF group

and 2.2 mg/L (IQR: 4.0) in the group without AF (p log CRP <0.01). Table 1 shows the baseline characteristics of the participants stratified by AF status and hs-CRP level greater or less than 3mg/L. Participants with hs-CRP level > 3mg/L were more likely to be women, black and have more comorbid condition like hypertension, diabetes, hyperlipemia, CHF, obesity and smokers.

The total follow up time to last in-home visit, death or stroke was 10,750 person-years in the AF group and 121,709 person-years in the non- AF group. During follow up of 8.3 years (median 5 years), 786 strokes occurred of which 130 occurred in the AF group and 676 in the group without AF. Stroke event rates were 12.1 and 5.6 per 1000 person-years in the AF and non-AF groups respectively (Table 2).

In a model adjusted for demographics and traditional stroke risk factors, a one unit change in hs-CRP was associated with increased risk for subsequent stroke in participants without AF (HR 1.01 95%CI 1.01, 1.02) but not in those with AF (HR 1.00 (95% CI 0.97-1.02) (Table 3). Stroke free survival curves in AF population with hs-CRP less vs greater than 3mg/L was not statistically significant (p 0.78) but appeared significant in population without AF (p<0.01) (Figure 1).

## **Discussion**

In this analysis from the REGARDS study, we examined the association between baseline hs-CRP with subsequent stroke in participants with AF as part of the REGARDS cohort compared to those without the diagnosis of AF. We found that participants with AF have higher hs-CRP levels at baseline. In addition, hs-CRP was not associated with subsequent stroke in the AF group after adjustment but there appears to be a strong association in the group without AF.

The temporal relationship between AF and inflammation, casual vs secondary, remains unclear, but there is considerable evidence in the literature on the association between inflammation and AF.<sup>32</sup> Cytokines, especially IL-6, illicit the expression of hs-CRP. As a dynamic marker of inflammation, hs-CRP provides a window to the inflammatory state of the individual which dramatically increase in response to tissue damage.<sup>33</sup> We have showed in this analysis that the group of participants with prevalent AF has higher levels of hs-CRP compared to the non-AF group which is consistent with previous studies in the literature.

Chronic elevation of baseline hs-CRP has been shown to be predictive of increased stroke risk.<sup>17-22</sup> A study from the Third National Health and Nutrition Examination Survey (NHANES) examined the association between CRP concentrations and self-reported past history of stroke among 8850 US men and women aged  $\geq 40$  years. The results revealed that higher concentration is associated with greater risk of stroke.<sup>34</sup> In the Framingham Study, elevated plasma CRP levels were shown to be significantly predictive of the risk of future ischemic stroke and TIA in the elderly independent of other cardiovascular risk factors.<sup>22</sup> In the current analysis, we showed that baseline hs-CRP is associated with increased risk of stroke in a well-represented biracial US population without AF and the relationship remained significant after adjustment for traditional stroke risk factors. The findings of this study support previous studies suggesting that elevated hs-CRP level may be a marker of increased risk of stroke.

The relationship between hs- CRP and future risk of stroke in the AF population was not significant in this analysis. It has been previously shown that patients with cardio-embolic stroke compared to other subtypes had significantly higher median

levels of multiple inflammatory markers like TNF-alpha, IL-6 and IL -1beta<sup>35</sup>. In fact, once an ischemic event transpire, the expression of inflammatory molecules and activation of quiescent inflammatory cells leads to inflammatory state for several months after initial insult<sup>36,37</sup>. In a study of 880 subjects investigating the relationship between hs-CRP and stroke risk, baseline hs-CRP levels were shown to be predictive of mortality and vascular death in atrial fibrillation, but not stroke.<sup>38</sup> Indeed, the timing inflammation may post date the ischemic insult. Furthermore, although AF itself may lead to an inflammatory state<sup>32</sup>, once an inflammatory state is achieved, the year to year within person variation of hs-CRP levels are similar to those in cholesterol and systolic blood pressure and the risk of future disease can be underestimated using a single measure at baseline.<sup>39</sup> Therefore, it is plausible that participants that suffer a stroke in the setting of known AF are previously in chronic inflammatory state which makes detection of association between inflammation and stroke difficult. In addition, most individuals with atrial fibrillation are more likely to have various comorbidities like elevated BP, BMI, diabetes and current smoking which further enhance the baseline inflammatory state.<sup>24</sup>

We acknowledge certain limitations of our study. Given that AF in our study was ascertained by two methods (self-report of a previous physician diagnosis and study scheduled ECG) which has been shown to have similar stroke predictive value<sup>40</sup>, some of AF cases may not been captured in this cohort at the time of baseline visit due to paroxysmal AF. Participants of the study that were unaware of AF status on enrollment but informed later and may have been started treatment including anticoagulation. Congestive heart failure in REGARDS was based on self-reported history of heart

failure symptoms including orthopnea and paroxysmal nocturnal dyspnea, which may induce some misclassification.

In conclusion, in this large prospective cohort of biracial US population, we found that hs-CRP was not significantly associated with stroke risk in population with AF. These findings suggest a limited value of hs-CRP in improving stroke risk stratification in pateints with AF. On the other hand, higher baseline levels of hs-CRP were significantly associated with stroke risk in population without AF which is consistent with previous studies.

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Disclosures

**None**

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**Table 1: Baseline characteristic of participants by baseline AF status and hs-CRP level**

Hs-CRP	- Atrial Fibrillation		+ Atrial Fibrillation	
	≤3.0 mg/l	>3.0 mg/l	≤3.0 mg/l	>3.0 mg/l
Total (n)	15124	10077	1299	1109
Total Stroke (n)	367	309	70	60
Age (+/-SD)	64.8 (+/- 9.5)	64.0 (+/- 9.1)	68.3 (+/- 9.7)	66.7 (+/- 9.6)
Black,%	35.4	49.5	29.9	43.6
Male, %	51.8	34.7	53.0	38.5
HTN,%	52.6	65.6	64.8	74.3
DM,%	17.3	25.1	22.2	28.9
HLD,%	33.3	30.3	43.3	39.3
BMI, Kg/m2 (+/-SD)	27.8 (+/-5.1)	31.4 (+/-7.0)	28.0 (+/- 5.5)	31.2 (+/-7.1)
Smoking (current and previous)	52.33	57.02	55.9	62.1
CHF (Orthopnea and PND)	2.9	5.7	9.5	16.4
CAD	15.4	15.9	34.3	37.3
Asprin, %	44.0	40.6	53.0	47.3
Coumadin, %	1.5	1.5	21.5	22.5

Hypertension (HTN), diabetes mellitus (DM), hyperlipidemia (HLD), body mass index (BMI), Congestive heart failure (CHF), Coronary artery disease (CAD)

**Table 2: Rate of stroke stratified by baseline AF status**

AF status	Persons	Person-Years (pr-yr)	Stroke (n)	Rate of Stroke
- AF	25,201	121,709	676	5.6 per 1000 pr-yr
+ AF	2,408	10,750	130	12.1 per 1000 pr-yr

**Table 3: Hazard ratio and 95% confidence interval of stroke associated hs-CRP by baseline atrial fibrillation (AF) status**

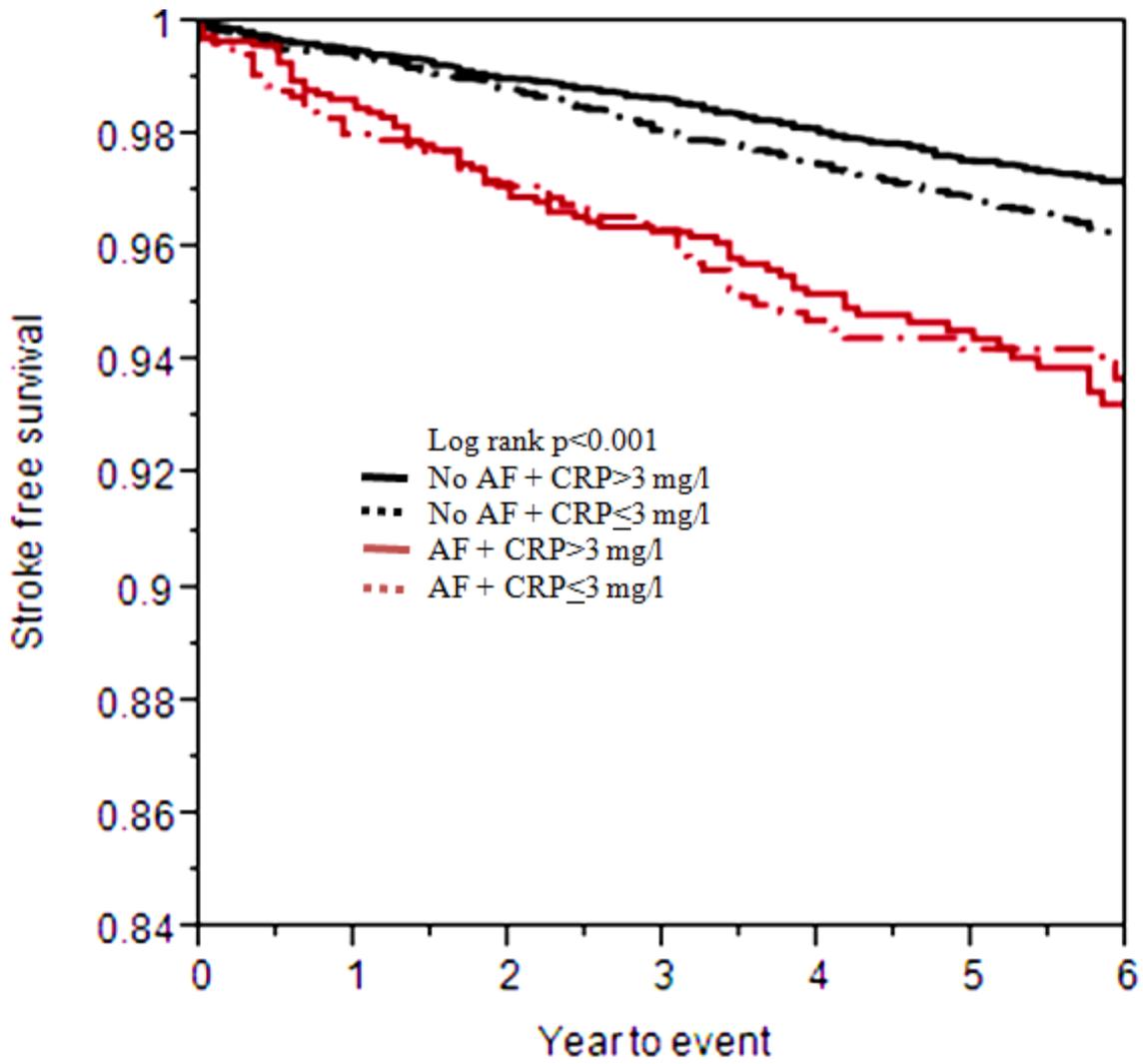
AF status	Model 1 (HR)*	Model 2 (HR) †	Model 3 (HR) ‡
- AF	1.012 (1.008-1.0153)	1.012 (1.008-1.0153)	1.011(1.006-1.015)
+ AF	0.996 (0.971-1.014)	1.001 (0.977-1.017)	0.998(0.973-1.016)

\*Model 1, unadjusted

† Model 2, adjusted for age, sex, and race

‡Model 3, Model 2 plus hypertension, diabetes mellitus, congestive heart failure, previous stroke/transient ischemia attack, and coumadin/asprin use

Figure 1: Kaplan Meier survival curves for event free survival by baseline hs-CRP and AF status



## CHAPTER 3

RELATIONSHIP BETWEEN INFLAMMATON, ATRIAL FIBROSIS, COAGULATION  
BIOMARKERS AND STROKE RISK IN ATRIAL FIBRILLATION: THE REASONS FOR  
GEOGRAPHIC AND RACIAL DIFFERENCES IN STROKE (REGARDS) STUDY

## Abstract

**Background and Purpose-** Atrial fibrillation (AF) increases the risk of stroke by 5-6 folds. Multiple biomarkers have been shown to be independently and strongly associated with increased risk of stroke in AF but their predictive ability has not been consistent. We hypothesized an association between multiple biomarkers and stroke in participants with AF in the REGARDS case cohort.

**Methods** – A total of 149 participants with AF (46.7 % African American, 58.7% female, age  $\geq$  45 years) of whom 71 had stroke were included in this analysis. Stroke cases were identified and adjudicated during 8.3 years follow up (median 5 years). AF was ascertained by ECG or self- reported history of previous physician diagnosis. Cox proportional hazard analysis was used to examine the risk of stroke in case cohort study in participants with AF.

**Results-** The AF group that suffered stroke had higher median levels of CRP, Cystatin C, fibrinogen, pro-BNP, Resistin, adiponectin, IL-6, D-Dimer, HGH, GFR and factor VIII activity level. Multivariate analysis comparing the upper to lower tertile showed NT-proBNP , D-Dimer, IL-6 and Cystatin C had significant HR after adjustment respectively: 4.39 (95% CI 1.25- 15.4), HR 4.47 (95% CI, 1.12-17.9), HR of 27.3 (95% CI, 1.80- 413) and HR of 3.39 (95% CI, 1.18- 9.74). The remaining biomarkers analyzed did not show significant HR.

**Conclusion-** After adjustments for multiple confounders, higher levels of NT-proBNP, D-Dimer, IL-6 and Cystatin C were significantly associated with increased risk of stroke in participants with AF.

**Keywords:** AF, stroke, biomarkers

## Introduction

Atrial fibrillation (AF) is the most common sustained cardiac rhythm disturbance encountered in clinical practice affecting 5% of individuals older than the age of 65<sup>1</sup>. Individuals afflicted with the arrhythmia have increased risk of morbidity and mortality adversely affecting quality of life<sup>2-4</sup>.

AF associated morbidity is predominantly related to 5-6 fold increased risk of ischemic stroke. Current recommended risk scores to predict stroke in AF are solely based on clinical variables. However, none of the available clinical schemas used in AF are fully exhaustive.<sup>5</sup> Recently, the CHA2DS2-VASc score was instituted as the preferred clinical score for predicting stroke in AF by the AHA/ACC guidelines<sup>6</sup>. Despite the ease of application, the currently available clinical scores only offer a modest discriminating value with C-statistic that range from 0.549 to 0.638<sup>7</sup>, where 1.0 is perfect discrimination and 0.5 is no better than random chance.

Biomarkers derived from the blood, such as markers of inflammation, coagulation, cardiovascular stress, cardiac and renal dysfunction may help refine risk assessment in AF patients. Most biomarkers have been assessed individually in different populations with different disease profiles and not collectively.<sup>8-13</sup> Furthermore, few of these markers have been studied in incident stroke in the setting of AF. A multimarker approach may provide better insight into the relationship between AF and its most dreaded complication stroke.

We examined the association between plasma levels of multiple biomarkers with stroke in individuals with AF in large population-based cohort of black and white Americans. The results of the analysis will provide insight into the relationship between

these novel markers and thromboembolism risk in AF and improve classification of patients in need of anticoagulation and ultimately reduce stroke burden.

## **Materials and Methods**

### **Study population**

The goals and design of the REGARDS study were published elsewhere <sup>14</sup>. REGARDS participants were enrolled in 2003-2007 from the 48 continental United States. The study was designed to investigate the causes of regional and racial disparities in stroke mortality, oversampling blacks and residents of the southeastern stroke belt region (North Carolina, South Carolina, Georgia, Alabama, Mississippi, Tennessee, Arkansas, and Louisiana). The cohort of 30,239 participants is 55% women, 45% men, 41% blacks, 59% whites, 56% living in the southeastern US stroke belt, and 44% living outside the stroke belt. Demographics, socioeconomic factors, medical history, and verbal informed consent were obtained by computer-assisted telephone interview. At an in-home examination, written informed consent, blood pressure, anthropomorphic measures, blood samples, ECG, and medication inventory were obtained. Study methods were reviewed and approved by the institutional review board at each study institution.

### **Covariates**

Standardized physical measures including height, weight and blood pressure were collected at the in-home physical examination. Demographics (age, sex, and race) were defined by self-report. Hypertension was defined as at least 1 of the following: systolic blood pressure  $\geq 140$  mm Hg, diastolic blood pressure  $\geq 90$  mm Hg or a self-report of currently taking medication to control blood pressure. Diabetes mellitus was defined as at least 1 of the following: fasting blood glucose  $\geq 126$  mg/dl, nonfasting

glucose  $\geq 200$  mg/dl, or a self-report of currently taking an oral hypoglycemic agent or insulin. Baseline glomerular filtration rate (GFR) was estimated using the Cockcroft–Gault and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. Previous CVD was defined by self-report (myocardial infarction or heart attack, coronary artery bypass surgery, coronary angioplasty, or stenting) or by ECG evidence of a previous myocardial infarction. Heart failure was defined as presence of orthopnea or paroxysmal nocturnal dyspnea.<sup>15</sup> Use of antihypertensive, lipid lowering, and antihyperglycemic medications, current warfarin treatment and aspirin use were defined using an inventory of current medications that was conducted during the in-home visits. The medication inventory was conducted before interpretation of the ECG and detection of AF.

### **Stroke Events**

Details on stroke events identification and adjudication have been previously published.<sup>16</sup> In summary, report of a possible stroke/transient ischemic attack or a positive response to the stroke symptoms on the Questionnaire for Verifying Stroke-Free Status<sup>17</sup>, resulting in hospitalization and follow-up generated a request for retrieval of medical records that were centrally adjudicated by a panel of stroke expert physicians. Incident stroke cases were included if they were reported by August 1, 2011, adjudicated by January, 2012, and met the WHO stroke definition or REGARDS clinical stroke definition.<sup>18</sup> Incident cases that were identified through death adjudication were included only if medical records were reviewed. Participants were not eligible to become incident stroke cases if at baseline they reported a history of physician-diagnosed stroke. Follow-up time for incident stroke was measured from the date of the in-home study visit until the date of incident stroke, date of death, or the last study

telephone contact prior to August 1, 2011. Of the 646 incident stroke cases, 573 were ischemic and 73 were hemorrhagic.

### **AF Diagnosis**

Information on AF ascertainment was previously published<sup>19,20</sup>. AF was diagnosed based on ECG that was recorded during the in-home visit by a healthcare professional. Staff members were trained in standard procedures with use of centrally trained supervisors, a web-based program, and continuous quality feedback to individual examiners. The ECGs were sent to a central ECG reading center at Wake Forest University where they were read, coded, and interpreted by electrocardiographers blinded to clinical data. Awareness of AF was defined as a positive response to the question, “Has a physician or a health professional ever told you that you had AF?” The telephone interview that established awareness of AF was conducted before the in-home visit where the ECG was performed.

### **Case–Cohort Sample**

We used a case–cohort study design with a mean follow-up of 5.4 years. Cases included 576 participants with incident ischemic stroke. The cohort random sample was selected using stratified sampling to ensure sufficient representation of high-risk groups. Participants were given a random number and divided into 20 strata based on age (45–54, 55–64, 65–74, 75–84, and ≥85 years), race, and sex. In each stratum, participants were randomly selected to fulfill the desired distribution: 50% black, 50% white; 50% women, 50% men; and age groups 45 to 54 (20%), 55 to 64 (20%), 65 to 74 (25%), 75 to 84 (25%), and ≥85 (10%) years. Of 1104 selected participants, we excluded 87 with previous stroke.

## Biomarkers

Participants contributed blood samples at the baseline in-home study visit. Fasting baseline blood samples stored using standardized methods. Plasma biomarkers comprised of adiponectin (assay range is 16-250,000 pg/ml; intra- and inter-assay CVs range from 1.4-7.9% and < 21%, respectively), Cystatin C, D-Dimer (normal range established during validation is 0.06-0.77 ug/ml; interassay CV ranges from 5 to 14%), fibrinogen (expected values in normal healthy individuals are 180 – 350 mg/dl; intra-assay CV ranges from 3% to 5%), Glomerular filtration rate (GFR), Human Growth Factor (HGF) (assay range for HGF is 19.2-50000 pg/ml; intra- and inter-assay CVs range from 1.4-7.9% and < 21%, respectively), Interleukin-6 (IL-6) (expected normal range per the manufacturer is 0.24 - 12.5 pg/mL; laboratory analytical CV for this assay is 6.3%), Interleukin-8 (IL-8) (assay range is 0.13-10000 pg/ml; intra- and inter-assay CVs range from 1.4-7.9% and < 21%, respectively), Leptin (assay range is 16 – 250,000 pg/ml; intra- and inter-assay CVs range from 1.4-7.9% and < 21%, respectively), Lipoprotein (a) (LP (a)) (expected values in normal healthy individuals range from <0.2-114.0 mg/dl; intra-assay CVs range from 1.8–4.1% and inter-assay CVs range from 2.0–5.3%), N-terminal pro- B-type natriuretic peptide (NT-proBNP) (expected values in normal healthy individuals are 125-450 pg/mL and detectable range is 5-35000 pg/mL; inter-assay CV provided by the manufacturer is less than 5%), resistin (assay range is 3.2 – 50,000 pg/ml; intra- and inter-assay CVs range from 1.4-7.9% and < 21%, respectively) and Factors VIII and XI Activity (expected normal range of activity is between 60% and 150% for Factors VIII and IX; assay CVs are 11% for Factor XI and 15% for Factor VIII).

## Statistical Analysis

Analyses were done using SAS 9.3 (Cary, NC). Frequency distributions of all variables were first inspected to identify anomalies and outliers possibly caused by measurement artifacts. Continuous data were described by their mean and SD and categorical data as proportions (percentage). Biomarkers of interest were displayed as means or proportions in the case-control sample. Differences among tertiles were compared by  $\chi^2$  tests or ANOVA using sampling weights. Cox proportional hazards analysis was used to examine the hazard ratios (HR) and 95% confidence interval (CI) for the association of incident stroke with biomarkers comparing lower tertile with the highest tertile in participants with AF. Independent associations of the biomarkers of interest with race were evaluated in a multivariable linear regression model including only factors significantly associated at  $P < 0.05$ . Participants without incident stroke were censored at death or last follow-up. All analyses were weighted using inverse probability weights to account for selection into the case cohort. The final model included history of stroke, CHF, hypertension, diabetes, CAD, age, race, sex.

## Results

During follow up of 8.3 years (median 5 years), total of 71 strokes occurred in 149 participants with AF in the REGARDS case cohort. Table 1 shows the baseline characteristics of the participants with AF included in the case cohort stratified by stroke status including median levels of the biomarkers used in the study. The AF group with stroke was older (72.1 y (+/- 8.4)), mostly male (56.5%), white (75.4%) and more likely to have HTN, DM, CHF and CAD. The AF group that suffered stroke had higher median levels of CRP, Cystatin C, fibrinogen, pro-BNP, Resistin, adiponectin, IL-6, D-Dimer, HGH, GFR and factor VIII activity level.

Cox proportional hazards analysis was used to examine the hazard ratios (HR) and 95% confidence interval (CI) for the association of stroke with biomarkers comparing lower tertile with the highest tertile in participants with AF. After adjusting for age, race, sex, CHF, hypertension, diabetes and CAD, participants with NT-proBNP in the top versus the bottom tertile had a hazard ratio of 4.39 (95% confidence interval 1.25- 15.4). Similar results were found comparing participants with the highest tertile to the lowest tertile hazard ratio of D-Dimer, IL6 and Cystatin C respectively: D-Dimer (HR) 4.47 (95% CI, 1.12-17.9), IL-6 (HR) of 27.3 (95% CI, 1.80- 413) and Cystatin C had a HR of 3.39 (95% CI, 1.18- 9.74). Resistin was approaching significance with HR of 3.13 (95% CI, 0.97-10.1). The remaining biomarkers analyzed in this study did not have statistically significant HR. The analyses were repeated including seven additional individuals with self-reported history of stroke and the results remained unchanged.

## Discussion

In this analysis, we examined the association between multiple baseline biomarkers and stroke risk in the REGARDS case cohort in participants with AF. Participants who suffered a stroke were more likely to be older, white and male with multiple traditional risk factors for stroke. The AF group that suffered stroke had higher median levels of CRP, Cystatin C, fibrinogen, pro-BNP, Resistin, adiponectin, IL-6, D-Dimer, HGH, GFR and factor VIII activity level.

The prevalence of AF is known to be higher in end stage renal disease patients compared to the general population and in fact AF prevalence increases as the glomerular filtration rate (GFR) decrease<sup>21-25</sup>. Furthermore, chronic kidney disease (CKD) confers a prothrombotic state and therefore represents risk factor for thromboembolic events in AF<sup>26-27</sup>. Despite that renal impairment is not represented in the current risk stratification schema to predict stroke in AF. Cystatin C is synthesised at constant rate in all nucleated cells and minimally influenced by disease states and therefore believed to be a more reliable marker of renal function than creatine which is used to calculate the GFR<sup>28-30</sup>. Participants with AF in the REGARDS case cohort who suffered a stroke compared to those free of stroke were noted to have increased risk of stroke as Cystatin C level increased (HR 3.47; CI (1.20-10.02)). Rising cystatin C levels were similarly independently associated with increased rates of stroke in the ARISTOTLE and RE-LY biomarker substudies.<sup>27-31</sup> However, GFR level in participants with AF who suffered a stroke compared to those free of stroke were not associated with increased risk of stroke unlike what was previously reported in the ATRIA study or ARISTOLE trial.<sup>26,27</sup>

BNP is a neurohormone secreted from atrial myocytes mainly due to increase wall tension and hemodynamic stress like in the case of AF<sup>32-33</sup>. It is synthesized as an inactive prohormone and cleaved into the bioactive hormone, BNP, and the inactive N-terminal fragment (NT-proBNP). The prognostic value of BNP and use in risk prediction in AF patients was first reported in 6189 patients with AF in the RE-LY biomarker study and in fact rising levels of NT-proBNP correlated with the risk of thromboembolic events<sup>28</sup>. The ARISTOTLE biomarker study further showed elevated risk of Ischaemic stroke and rising NT-proBNP Levels<sup>34</sup>. In the REGARS substudy, similar findings were observed. There was a significant increase in risk of stroke in AF population with increasing level of BNP when the lower tertile was compared to highest tertile (HR 4.19; CI (1.20-14.68)). This may be explained by increasing stress in the fibrillating atria leading to inflammatory response and endothelial activation leading to thrombus formation which is a known risk factor for stroke in AF.

The prothrombotic state in atrial fibrillation has been well described in the literature<sup>35-38</sup>. D-dimer originates from the formation and lysis of cross-linked fibrin and denotes activation of coagulation and fibrinolysis. In the RE-LY biomarker study, baseline D-dimer level was strongly associated with risk of stroke, cardiovascular death, and major bleeding independent of established risk factors for stroke including the CHADS2 variables<sup>39</sup>. Similarly, we showed that increasing levels of D-Dimer are significantly associated with increased risk of stroke in the AF population that suffered a stroke (HR 4.48; CI (1.12-17.94)).

There is growing evidence that inflammation may be associated with AF<sup>40-43</sup>. Inflammatory mediators may confer a prothrombotic state by promoting endothelial damage and platelet activation in patients with AF, thus linking inflammation and

thrombosis. IL-6 is a pleiotropic cytokine with a variety of biological activities, including mediation of proinflammatory responses and stimulates the synthesis of several acute phase reactants like CRP. Conway *et al.* reported the association between IL-6 and a composite outcome of stroke and death in a small AF population <sup>44</sup>. It was shown later in the RE-LY biomarker substudy that there is an independent association between IL-6 and stroke or systemic embolic events in large number of AF patients <sup>45</sup>. In the current analysis, participants with the top tertile levels of IL-6 compared with the bottom tertile, stroke risk in adjusted analysis was significant confirming the intimate relationship between inflammation and thrombosis.

The strength of this study is the large cohort size of blacks and whites followed prospectively after extensive baseline data collection. The events were carefully adjudicated with only 12.7% cumulative dropout as of January 2011. We acknowledge certain limitations of our study. Given that AF in our study was ascertained by two methods (self-report of a previous physician diagnosis and study scheduled ECG) which has been shown to have similar stroke predictive value <sup>46</sup>, some of AF cases may not been captured in this cohort at the time of baseline visit due to paroxysmal AF. Results only generalize to black and white Americans. Markers were measured once and therefore regression dilution bias could not be controlled although this would bias the result toward the null.

In conclusion, among patients with AF who suffered a stroke, several inflammatory and thrombosis biomarkers were elevated compared to patients with AF that are free of stroke. Elevations of pro-BNP, D-Dimer, IL6, and Cystatin C levels are independently related to increased risks of stroke. These cardiac biomarkers may be useful for improving risk prediction in AF beyond currently used clinical variables.

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## **Disclosures**

None

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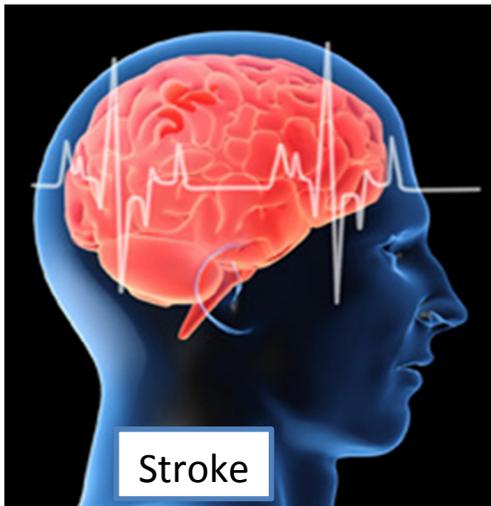
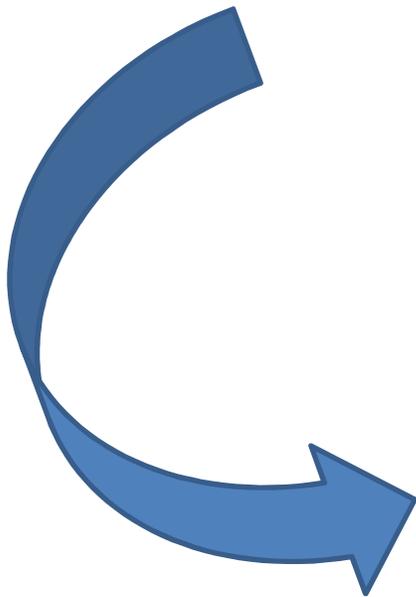
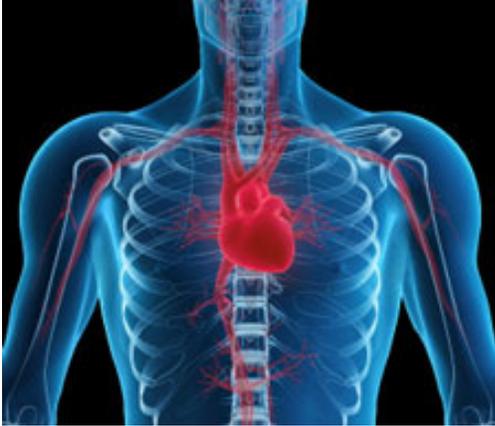
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**Table 1: Baseline Characteristics stratified by Stroke Status**

	Stroke Free (n= 78)	Stroke (n=71)
<b>Age, y (+/-SD)</b>	<b>69.3 +/- 12.4</b>	<b>72.1 +/- 8.4</b>
<b>White,%</b>	<b>53.3</b>	<b>75.4</b>
<b>Male, %</b>	<b>41.3</b>	<b>56.5</b>
<b>HTN,%</b>	<b>56.0</b>	<b>75.4</b>
<b>DM,%</b>	<b>25.3</b>	<b>26.1</b>
<b>HLD, %</b>	<b>52.6 +/- 18.3</b>	<b>48.2 +/-17.2</b>
<b>CHF (Orthopnea and PND)</b>	<b>12.0</b>	<b>14.5</b>
<b>CAD</b>	<b>29.3</b>	<b>53.7</b>
<b>Body Mass Index, Kg/m2</b>	<b>27.7 +/- 5.56</b>	<b>28.23 +/- 6.00</b>
<b>Smoking, %</b>	<b>20.5</b>	<b>11.3</b>
<b>CRP, mg/dl</b>	<b>4.24 +/- 5.74</b>	<b>4.45 +/- 6.00</b>
<b>Biomarkers</b>		
<b>Cystatin C (mg/L)</b>	<b>0.99</b>	<b>1.1</b>
<b>NT-proBNP (pg/ml)</b>	<b>124.25</b>	<b>383.8</b>
<b>Lipoprotein(a) (Lp(a)) (mg/dl)</b>	<b>0.24</b>	<b>0.22</b>
<b>Resistin (pg/ml)</b>	<b>23.67</b>	<b>27.65</b>
<b>Adiponectin (ug/ml)</b>	<b>8.89</b>	<b>12.86</b>
<b>fibrinogen (mg/dl)</b>	<b>402.5</b>	<b>417</b>
<b>Interleukin-6 (IL-6) (pg/ml)</b>	<b>2.92</b>	<b>3.77</b>
<b>Interleukin-8 (IL-8) (pg/ml)</b>	<b>2.82</b>	<b>2.82</b>
<b>D-Dimer (ug/ml)</b>	<b>0.41</b>	<b>0.57</b>
<b>Factor VIII, % activity</b>	<b>124.25</b>	<b>132.33</b>
<b>Factor IX, % activity</b>	<b>100.96</b>	<b>95.28</b>
<b>Leptin (ug/ml)</b>	<b>19.39</b>	<b>13.96</b>
<b>Human Growth Factor (HGF) (pg/ml)</b>	<b>307.47</b>	<b>363.58</b>

**Figure 1: Biomarkers Studied**



- *Kidney function*
    - *GFR, Cystain C*
- 
- *Cardiac Myocyte strain*
    - *NT-proBNP*
  - *Inflammation*
    - *IL6, IL8, IL10, resistin, adiponectin*
  - *Thrombosis*
    - *D-Dimer, Fibrinogen, factor IX, Fator VIII*
  - *Atrial Fibrosis*
    - *HGF, Leptin, LPA*

**Table 2: Hazard Ratio (95% Confidence Interval) of AF Associated Stroke by Baseline Biomarkers Levels**

Biomarkers Tertiles			
	1	2	3
BNP	Reference	1.26 (0.29, 5.52)	4.39 (1.25, 15.4)
Resistin	Reference	1.13 (0.35, 3.64)	3.13 (0.97, 10.1)
IL10	Reference	1.74 (0.44, 6.83)	1.34 (0.37, 4.92)
Adiponectin	Reference	1.76 (0.35, 8.81)	2.57 (0.40, 16.4)
Leptin	Reference	0.21 (0.07, 0.64)	0.71 (0.19, 2.70)
Lpa	Reference	0.86 (0.26, 2.87)	1.03 (0.28, 3.77)
Fibrinogen	Reference	0.49 (0.16, 1.48)	1.45 (0.40, 5.22)
D-Dimer	Reference	2.27 (0.55, 9.31)	4.47 (1.12, 17.9)
IL6	Reference	10.5 (0.95, 115)	27.3 (1.80, 413)
IL8	Reference	0.54 (0.16, 1.84)	0.62 (0.18, 2.13)
Factor VII	Reference	0.78 (0.25, 2.44)	1.45 (0.47, 4.47)
Factor IX	Reference	1.27 (0.41, 3.91)	2.18 (0.59, 8.02)
Human Growth Factor	Reference	0.52 (0.15, 1.77)	1.71 (0.56, 5.22)
Cystatin	Reference	1.60 (0.46, 5.50)	3.39 (1.18, 9.74)
GFR (Glomerular Filtration Rate)	Reference	0.64 (0.23, 1.77)	0.30 (0.08, 1.13)

Final model adjusted for age, race, sex, congestive heart failure (CHF), hypertension, diabetes and coronary artery disease (CAD)

## CURRICULUM VITAE

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### PROFESSIONAL LICENSURE AND CERTIFICATION:

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2001 Top ten graduates (Number six) among the department of Chemistry, University of California, San Diego  
2001 Excellence in the presentation of scholarly paper by the faculty mentor program, University of California, San Diego  
2001 Cum laude honors, University of California, San Diego  
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**PUBLICATIONS:**

O'Neal WT, Efird JT, **Dawood FZ**, Yeboah J, Alonso A, Heckbert SR, Soliman EZ. Coronary Artery Calcium and Risk of Atrial Fibrillation (from the Multi-Ethnic Study of Atherosclerosis). *Am J Cardiol.* 2014 Sep 16.

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### **Abstracts**

Atrial fibrillation prevalence and clinical correlates among diverse Hispanic/Latinos: The Hispanic Community Health Study / Study of Latinos (HCHS-SOL). AHA Epi 2015

Atrial Fibrillation as Risk Factor for Myocardial Infarction in the REGARDS cohort. Poster Presentation. ACC 2013

Correlation of the level of hs-CRP in atrial fibrillation patients in the REGARDS cohort who suffered ischemic stroke. Poster Presentation. AHA 2012

Inspection of the rate determining steps in the folding pathway of Interleukin- 1B via stopped flow fluorescence and equilibrium denaturation. Oral Presentation. Faculty mentor program at the University of California; San Diego, CA. 2011

Unusual Presentation of Chest Wall Abscess Following Acupuncture. Poster Presentation. NC ACP meeting 2010.

Determination of the topology of Leptin dimer using Fluorescence Resonance Energy Transfer (FRET). Oral Presentation. NSF Research Day, Santa Cruz, Ca (Northern California Conference); Santa Cruz, CA. 2000

### **Ongoing Research Projects**

Multiple Biomarkers and Incident Stroke in Participants with AF: The Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study

Atrial fibrillation Prevalence and Clinical Correlates Among Diverse Hispanic/Latinos: The Hispanic Community Health Study / Study of Latinos (HCHS-SOL)

Correlates of Bradycardia and its Impact on Incident CVD: The Multi-ethnic Study of Atherosclerosis (MESA)

The Efficacy and Safety of Transvenous Pacing: The Wake Forest University Experience

Reference values of P-wave indices, prognostic significance of PR prolongation and heart rate adjusted PR in the resting 12-lead electrocardiogram: The Multi-ethnic Study of Atherosclerosis (MESA)

High Sensitivity C-Reactive Protein and Risk of Stroke in Atrial Fibrillation: The Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study

Comparison of Novel Risk Markers for the Improvement of Risk Assessment by the New Pooled Atherosclerotic Cardiovascular Disease risk Tool: The Multi-ethnic Study of Atherosclerosis (MESA)