ARIC Manuscript Proposal #1994

PC Reviewed: 9/11/12	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1. a. Full Title: Association of white blood cell count and differential with the incidence of atrial fibrillation: the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): WBC and AF in ARIC

2. Writing Group:
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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. JM **[please confirm with your initials electronically or in writing]**

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3. Timeline:

Data analysis: 1-2 months from manuscript approval date.
First draft of the manuscript: 3-4 months from the manuscript approval date.

4. Rationale:

Atrial fibrillation (AF) continues to be a growing health concern in the United States. Over two million individuals are estimated to be currently affected by AF, and its prevalence is anticipated to double by 2050.[1] In addition to being the most frequently observed cardiac arrhythmia in clinical practice, AF has been associated with increased risk of cardiovascular disease (CVD), heart failure (HF), stroke, and overall mortality.[2-4] Numerous research studies have investigated prospective AF risk factors to gain a better understanding of its causation and prediction. After accounting for one or more borderline or elevated risk factors, though, a considerable proportion of the attributable risk of AF (44%) remains unexplained.[5]

Systemic inflammation and its association with AF has been examined previously through markers such as C-reactive protein and white blood cell (WBC) count.[6,7] Specifically considering WBC as a biomarker, an elevated count during the perioperative period was identified as being predictive of postoperative AF.[8-11] Another small cohort study found an association between an increased WBC count with recurrent AF after pulmonary vein isolation.[12] The association between WBC count and AF in the general population has been examined in a subset of the Framingham Heart Study. Among 936 eligible participants followed up for a maximum of 5 years, increased WBC was associated with increased risk of AF. However, this association was largely attenuated after additional follow up.[7] We propose to address the same hypothesis in the Atherosclerosis Risk in Communities (ARIC) Study including a larger sample size and extended follow-up, which will allow us to estimate the association in subgroups by race, sex, and age. Also, we will prospectively explore the association between WBC differential count and AF incidence. Granulocyte count has been linked to increased incidence of other CVD,[13] and there is evidence to suggest that an enzyme abundantly produced by neutrophils called myeloperoxidase may be involved in the development of atrial fibrosis, resulting in an increased risk of AF.[14,15] Therefore, WBC differential counts might show different associations with the risk of AF.

5. Main Hypothesis/Study Questions:

Aim #1: To determine if WBC count is associated with the incidence of AF in the ARIC study.

Aim #2: To determine if WBC differential counts are associated with the incidence of AF in the ARIC study.

As a secondary aim, we will explore whether race and sex modify the association of WBC count and WBC differential counts with AF risk.

We hypothesize that individuals with higher WBC count will have an increased risk for AF, and this association will be due to an increased risk of AF associated with higher levels of granulocytes (mostly neutrophils).

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodological limitations or challenges if present).

Study design:
A follow-up data analysis will be performed utilizing longitudinal data from the ARIC cohort, using visit 1 as baseline.

Inclusion/exclusion criteria:
We will exclude individuals (1) with prevalent AF or atrial flutter at baseline based on electrocardiogram (ECG), (2) missing baseline ECG data, (3) missing baseline WBC data, (4) missing other covariates, (5) with a race other than white or African-American, and (6) non-whites from the Minnesota and Washington County sites.

Variables of interest:

Main outcome of interest: Atrial fibrillation incidence
The time to incident AF cases from baseline through December 31, 2009, will be the main outcome variable. Incident AF cases were ascertained from three sources: ECGs completed during the study exams, ICD-9 codes of 427.31 or 427.32 from hospital discharges, and death certificates that include AF as a cause of death (ICD-9 code 427.3 or ICD-10 code I48). AF incidence date will be defined as the date of the first ECG showing AF, the first hospital discharge date for an AF or atrial flutter diagnosis, or date when death occurred due to AF, whichever occurred first.[16]

Main independent variables of interest: White blood cell count and differential
In the ARIC study, WBC count and differential were assessed through laboratory tests at visit 1. WBC differential counts measured at baseline include granulocytes (neutrophils, eosinophils, and basophils), lymphocytes and monocytes.

Covariates
From visit 1, other measured covariates to be included in the analysis are age, gender, race, study site, body mass index (BMI), height (because this variable is a strong predictor of AF independently of BMI), drinking status, diabetes mellitus, educational level, smoking status and cigarette-years, systolic blood pressure, use of medications (antihypertensive and steroids), chronic obstructive pulmonary disease (COPD) through lung function (forced expiratory volume in 1 second [FEV_1] and forced vital capacity [FVC]), and a history of HF, myocardial infarction (MI), and stroke.

Statistical analysis:
Cox proportional hazards models will be used to determine the association between WBC and incident AF. Initially, we will explore the shape of the association of WBC count and differential with AF risk using restricted cubic splines. Log transformations will be done if necessary. If appropriate, WBC count and differential will be divided into ranked quartiles. We will also assess linear associations based on the spline model. The following models will be used to analyze the WBC-AF association:

- Model 1: adjustment for age, gender, race, and ARIC study site
- Model 2: Model 1 + adjustment for BMI, height, drinking status, diabetes mellitus, educational level, smoking status and cigarette-years, systolic blood pressure, COPD, and use of medications (antihypertensive and steroids).
- Model 3: Model 2 + history of heart failure, MI, and stroke
- Model 4: Model 3 + incidence of heart failure, MI, and stroke as time-dependent covariates

Multiplicative effect modification will also be evaluated by age, gender, and race conducting stratified analysis and including multiplicative terms between the effect modifier and WBC measures in the models.

We expect to include more than 1700 incident events of AF, which will provide sufficient power to study the association of WBC count and differential with AF risk in the entire sample. However, limited power might exist to study race-specific associations, particularly in African Americans, and the WBC-AF association by the three different AF ascertainment sources.

Strengths and limitations:
Strengths of the study include the large sample size and power to measure associations between WBC and AF and the sizable sample of African Americans to evaluate risk factors of interest in relation to AF. However, there are a couple of limitations. Although hospital discharge codes being used for identifying incident AF cases have shown to be valid,[16] there is some likelihood of AF cases being missed in outpatient settings. In addition, there may be some misclassification of the WBC exposure since there is no follow-up information on WBC count and differential after visit 1. As a result, if the WBC measures happened to change over time, there is no additional information to examine such changes from follow-up data.

7. a. Will the data be used for non-CVD analysis in this manuscript?
___ Yes _X_ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used?
____ Yes ____ No
(This file ICTDER03 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8. a. Will the DNA data be used in this manuscript? ____ Yes _X_ No

b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"?
____ Yes ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php

_X_ Yes, no overlap found. _____ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

No previous manuscript proposals in ARIC have specifically examined the association between WBC count/differential and AF. Other ARIC manuscripts have explored the association between WBC and CVD:

#709: WBC, CHD, and Stroke
#1504: WBC, CRP, and HF
#1838: WBC and Ischemic Stroke

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? _X_ Yes ____ No

b. If yes, is the proposal
__ A. primarily the result of an ancillary study (list number*)
X B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* 2008.12)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

References:

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7. Rienstra M, Sun JX, Magnani JW, et al. White blood cell count and risk of atrial fibrillation (from the Framingham Heart Study). *Am J Cardiol*. 2012; 109(4):533-537.
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13. Bekwelem W, Lutsey PL, Loehr LR, et al. White blood cell count, C-reactive protein, and incident heart failure in the Atherosclerosis Risk in Communities (ARIC) Study. *Ann Epidemiol*. 2011; 21(10):739-748.
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15. Friedrichs K, Baldus S, Klinke A. Fibrosis in atrial fibrillation: role of reactive species and MPO. *Front Physiol*. 2012; 3(214):1-14.
16. Alonso A, Agarwal SK, Soliman EZ, et al. Incidence of atrial fibrillation in whites and African-Americans: the atherosclerosis risk in communities (ARIC) study. *Am Heart J*. 2009; 158:111-117.