ARIC Manuscript Proposal # 1206

PC Reviewed: _12/_19/06	**Status: __A_**	**Priority: _2__**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Association of established risk factors with blood platelet and monocyte cell-markers and cell aggregates (ARIC MRI)

b. Abbreviated Title (Length 26 characters):
Risk factors & cell markers

2. Writing Group:
Writing group members:
Aaron Folsom, Nena Matijevic, Diane Catellier, Eric Boerwinkle

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

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3. Timeline:
We hope to have a draft manuscript by March 2007/

4. Rationale:
The aim of this paper is to report the relation between the established risk factors for atherosclerotic disease and cell markers expressed by circulating blood platelets and leukocytes in the carotid MRI study.

Immune and inflammatory mechanisms are considered to play a key role in the pathogenesis of atherosclerosis. Many inflammatory blood and vascular cell types and their activation markers play a role in the initiation, progression, and all stages of atherosclerosis development. Cell activation and cell-cell interactions result in the production of a cascade of cytokines, chemokines and other proinflammatory molecules that contribute to the disease.

Multiple studies have demonstrated a role of platelets as inflammatory cells; several platelet derived factors, both membrane bound and soluble may be involved in the inflammatory interaction between platelets, leukocytes, and endothelial cells. The platelet-leukocyte cross-talk involves a wide range of mediators such as chemokines, adhesion molecules, reactive oxygen species, and cytokines. Multiple clinical studies reported an association of platelet and leukocyte markers with atherosclerotic disease.

In the carotid MRI study, we measured the expression of a number of blood platelet and leukocyte markers and cell aggregates:
Platelet membrane glycoproteins IIb (CD41) and IIIa (CD61); platelet activation markers P-selectin (CD62P) and CD40L (CD154); platelet-platelet aggregates (PPA); platelet-leukocyte aggregates (platelet-monocyte; platelet-lymphocyte; platelet-granulocytes); monocyte lipopolysacharide (LPS) receptor (CD14); monocyte membrane expression of toll-like receptors TLR2 and TLR4; leukocyte membrane expression of CD45 (leukocyte common antigen) and PSGL-1 (P-selectin glycoprotein ligand-1; CD162); intracellular levels of the two leukocyte enzymes: myeloperoxidase (MPO) and cyclooxygenase-2 (COX-2).

In order to estimate the ability of peripheral blood circulating cellular markers to reflect the inflammatory alterations of the atherosclerotic plaques in carotid atherosclerosis, it is important to analyze the influence of traditional risk factors on the expression of cellular markers. It is important to better understand if there is a potential for confounding between traditional risk factors and cellular markers with respect to atherothrombotic disease. The potential association of cell markers with constitutional, lifestyle and biochemical characteristics of participants must be taken into account when analyzing the role of any of these cellular factors in the development and progression of atherosclerotic diseases.

5. Main Hypothesis/Study Questions:
Are established risk factors associated with expression of blood cell markers in the carotid MRI study?

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 methodologic limitations or challenges if present).

This is a cross sectional analysis of the carotid MRI data performed at the CSCC.

Exclusions: missing covariates, any cell marker that proved not to be reliable (i.e., $r<0.6$).

Independent variables: basic risk factors (age, race, gender, LDL-C, HDL-C, lipid med use, systolic BP, antihypertensive med use, diabetes, obesity, cigarette smoking status, alcohol intake, physical activity, BMI, waist to hip ratio, and CRP). All will be from the carotid MRI visit.

Covariates: regular aspirin use, coumadin, others still being considered

Dependent variables: platelet and leukocyte markers.

Analysis: Because of the design of the carotid MRI study, weighted analyses for complex surveys will be used throughout. The first step will be to review the QC data and remove the assays that are not reliable. Next we will examine the mean values and distributional characteristics of the dependent variables. Next, we will look at correlations among the numerous cell marker variables. If many are highly correlated, a smaller subset might be identified to serve as independent variables.

T tests, ANOVA, or chi-square tests will be used to assess associations of age, sex, or race with dependent variables. If any of these sociodemographic variables are significantly related to cell marker variables, we will model the association of other risk factors with cell markers using linear regression with adjustment for these factors. This modeling approach will also be used to control for potential confounding factors (e.g., aspirin use, coumadin) as needed. The independent variable of interest may be model using a regression spline or smoothing spline if its relationship to the cell marker is non-linear.
The regression models may be extended to include all predictors found to be significant in univariate analyses described above, to determine which among them are independent predictors of the cell markers.

The challenge of this analysis is the many dependent variables and multiple independent variables, which is probably too much for one paper, unless few associations are evident. We will split out papers as seems appropriate. One logical split is by 1) Platelet markers, 2) Monocyte markers, 3) Cell-cell interactions (platelet-platelet and platelet-leukocyte aggregates).

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

b. If Yes, is the author aware that the file ICTDER02 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 ICTDER02 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
__xx__ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 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

__xx____ Yes _____ 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)?

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

I assume ARIC MRI is not ancillary.

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

*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.