ARIC Manuscript Proposal # 1207

PC Reviewed: _12/19_/06	**Status: __A__**	**Priority: _2__**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Association of monocyte markers with peripheral arterial disease (PAD)

b. Abbreviated Title (Length 26 characters):
Monocytes & PAD

2. Writing Group:
Writing group members:
Nena Matijevic, Aaron Folsom, Diane Catellier, Gerardo Heiss,…others

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _____ **[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 various cell markers expressed by circulating blood monocytes and peripheral arterial disease (PAD) in participants of the ARIC 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.

PAD is an important manifestation of systemic atherosclerosis. It is associated with elevated risk for cardiovascular and cerebrovascular events, including CHD and stroke. An ankle-brachial index (ABI) less than 0.9 serves as the diagnostic tool used to define PAD. PAD is strongly associated with inflammatory markers. Circulating monocytes and tissue macrophages are a major source of inflammatory cytokines. WBC count has been consistently associated with cardiovascular end points. Results from the NHANES including 3949 individuals showed that monocytes are the only WBC subtype significantly and independently associated with PAD, but this study was limited to the monocyte count only.

In the carotid MRI study, we measured numerous markers expressed by circulating monocytes, and we propose to analyze the association of monocyte markers with presence/severity of reduced ankle-brachial blood pressure index (ABI), a marker of PAD. We want to test the hypothesis that circulating monocyte markers reflect the extent of vascular atherosclerotic state.

Blood monocyte cell markers measured in the ARIC Car MRI study are:
Monocyte lipopolysacharide (LPS) receptor (CD14); monocyte membrane toll-like receptors TLR2 and TLR4; leukocyte membrane receptors CD45 (leukocyte common antigen) and PSGL-1 (P-selectin glycoprotein ligand-1; CD162); intracellular levels of two enzymes: myeloperoxidase (MPO) and cyclooxygenase-2 (COX-2), and heterotypic platelet-monocyte aggregates (PMA).

Toll-like receptors (TLRs) are expressed preferentially on monocytes/macrophages which play a critical role in recognizing microbial pathogens and produce proinflammatory cytokines. Monocyte CD14 and TLR-4 are two components of the LPS (endotoxin) receptor complex which recognizes gram-negative bacteria and their toxins, has also been reported to be a receptor for endogenous ligands such as fibrinogen, fibronectin, heat-shock protein. TLR-2 responds to various bacterial products including lipoproteins and gram-positive bacterial peptidoglycan. PSGL-1 (CD162) is constitutively expressed on circulating leukocytes; it serves as the counter receptor for P-selectin and possibly E- and L-selectin; it mediates rolling of leukocytes during inflammation and thus plays a pivotal

role in hemostasis and inflammation. Until recently, PSGL-1 was considered not to be regulated upon activation. However, lately, neutrophils and monocytes were found to down-regulate PSGL-1 upon stimulation with proinflammatory substances. Myeloperoxidase (MPO) is a leukocyte enzyme that plays a role in host defense by generating reactive oxidants. It is synthetized exclusively by normal neutrophil and monocyte precursor cells and is released upon cell activation and degranulation. MPO also promotes oxidative damage of host tissues at sites of inflammation, including atherosclerotic lesions. Cyclooxygenase-2 (COX-2) is the key enzyme controlling eicosanoid production in atherosclerosis and other inflammatory syndromes. COX-2 mediated prostaglandin production by activated macrophages is associated with inflammation and atherosclerosis.

5. Main Hypothesis/Study Questions:
Monocyte markers are significantly and independently associated with PAD.

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.

Dependent variable: ABI (low vs normal; high ABI will be examined too, but it is not clear whether this is abnormal or not. Later analyses probably will exclude high ABI).

Independent variables: monocyte markers

Covariates: 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 MRI visit.

Analysis: The first step will be to present the mean values and distributional characteristics of the independent 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.

Next, we will look at mean values of independent variables by categories of independent variables; we will correlate the monocyte markers in relation to the ABI index, ie. Low (<0.9) vs. normal (0.9-1.3) vs. high (>1.3).

Finally, we will use a multivariable logistic model, to determine independent predictors of the low ABI vs normal.

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.