1.a. Full Title: Peripheral blood monocyte myeloperoxidase (MPO) and cyclooxygenase-2 (COX-2) levels and carotid artery plaque presence/progression (ARIC CAR MRI Study)

b. Abbreviated Title (Length 26 characters):
Monocyte MPO, COX2 and MRI

2. Writing Group:
Writing group members:
Nena Matijevic, Kenneth Wu, Willa Wang, Aaron Folsom, Lloyd Chambless/Diane Catellier (or else from the CC) Brad Astor, Richey Sharrett, Christie Ballantyne.

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

First author: Matijevic

Address: Nena Matijevic, Ph.D.
Assistant Professor of Medicine
Internal Medicine, Division of Hematology
Flow Cytometry Lab (M.S.B. 5.436)
The University of Texas Health Science Center at Houston
6431 Fannin, M.S.B. 5.240
Houston, Texas 77030 - 1503. U.S.A.

Phone: 713.500.6807 Fax: 713.500.6810

E-mail: nevenka.matijevic-alekic@uth.tmc.edu
3. Timeline:
We hope to have a draft manuscript by March 2007.

4. Rationale:
In order to estimate the ability of peripheral blood circulating cellular markers to reflect the inflammatory alterations of the atherosclerotic plaques in carotid atherosclerosis, we will analyze the relation between the whole blood monocyte intracellular levels of the MPO and COX-2 and atherosclerotic plaque presence/progression in carotid artery.

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 lesion development. Cell activation and cell-cell interactions result in the production of a cascade of cytokines, chemokines, adhesion molecules, reactive oxygen species and other proinflammatory molecules that contribute to the disease. A variety of endogenous agents are involved in the regulation of the cytokine network. Eicosanoids and reactive oxygen intermediates have an especially great impact on the regulation of cytokine production providing an important link between innate and adaptive immunity.

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. Immunohistochemical studies have demonstrated the presence of MPO in human atherosclerotic lesion. MPO is elevated in the circulation of people at risk for cardiovascular disease. MPO plasma levels were reported to be able to identify patients at risk for cardiac events in the absence of myocardial necrosis. It was suggested that MPO might be potentially useful for the risk stratification for CVD status and outcome.

Major evidence for MPO-mediated oxidative modification of lipoproteins in the artery wall came from studies on low density lipoprotein. Recently, it has been shown that MPO-catalyzed oxidation products are highly enriched in circulating HDL from individuals with cardiovascular disease where MPO concentrations are also increased. MPO was found to be associated with HDL extracted from human atheroma. All these MPO-generated products may contribute to generating dysfunctional HDL and related enzymes and contribute to atherosclerosis.

Cyclooxygenase-2 (COX-2) is the key enzyme controlling eicosanoid production in atherosclerosis and other inflammatory syndromes. The pathophysiologic role of COX-2 and prostanoids in atherothrombosis is recognized to be very complex. COX-2 mediated prostaglandin production by activated macrophages is associated with inflammation and atherosclerosis. Increased proportion of circulating monocytes expressing COX-2 was reported in insulin-dependent diabetes patients. Immunohistochemical studies have demonstrated the presence of COX-2 in human atherosclerotic lesions. Macrophages of...
the shoulder plaque region contain most of the COX-2 protein within the lesion. Patients with carotid atherosclerosis were reported to have an elevated expression of COX-2 simultaneously in the peripheral blood mononuclear cells as well as in the vulnerable plaque regions.

The CD45 is leukocyte common antigen expressed on all nucleated hematopoietic cells, but the density of CD45 molecule on these cells is different. It has been recognized as an important player in regulating signaling in lymphocytes. However, its role is still poorly understood in terms of its regulation and function. Recently a novel activity of anti-CD45 antibody with reverse modulation on cyclooxygenase/lipoxygenase pathway was reported by affecting arachidonic acid metabolism.

In this study, we measured MPO and COX-2 levels in the peripheral blood monocytes of the ARIC CAR MRI study participants by flow cytometry. Whole blood leukocytes were labeled with pan-leukocyte monoclonal antibody to the surface receptor CD45, monoclonal antibody to the monocyte LPS receptor CD14, and monoclonal antibodies to the intracellular MPO and COX-2.

5. **Main Hypothesis/Study Questions:**
   a) Peripheral blood monocyte MPO and COX-2 reflect the inflammatory alterations of the atherosclerotic plaques.
   b) Patients with increased wall volume have increased level of MPO and COX-2 in the circulating monocytes.
   c) Monocyte MPO and COX-2 levels are independently associated with carotid artery plaque presence in atherosclerotic patients.
   d) Monocyte MPO and COX-2 levels may serve as a novel marker to identify the individuals at increased risk of plaque vulnerability.

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).**

CD45, CD14, MPO, COX-2. We will analyze the percentage of cells expressing the markers and the relative levels of MPO and COX-2 expression (mean or median fluorescence intensity). In addition, we will analyze and compare the levels (MFI) of the CD14 expression in monocytes, and CD45 expression on monocytes and granulocytes and correlate it with intracellular enzymes levels.

Exclusions: missing covariates, any cell marker that proved not to be reliable.

MRI variables: wall thickness, plaque presence/absence, lipid core volume, fibrous cap thickness

Independent variables: CD45 (MFI), CD14 (MFI), MPO (MFI), COX-2 (% MFI)
The association of the independent variables with the MRI dependent variables (wall volume, lipid core volume, fibrous cap thickness, etc) will be assessed by linear or logistic regression (the latter for any categorical MRI variables), adjusting for age, race, sex, and additional by other covariates listed below.

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.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes ___ 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 ___ 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.csec.unc.edu/ARIC/search.php

     ____ 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 ___ No

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.