ARIC Manuscript Proposal # 1335

1. a. Full Title: Platelet and leukocyte markers and plasma MMPs (ARIC CAR MRI Study)

   b. Abbreviated Title (Length 26 characters):
   Flow cytometry and MMPs

2. Writing Group:
   Writing group members:
   Nena Aleksic, Ron Hoogeveen, Christie Ballantyne, Woody Chambless, others welcome…

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

First author:

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Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author):
Address:
3. **Timeline:**
We hope to have a draft manuscript by April 2008.

4. **Rationale:**
Many inflammatory blood and vascular cell types and their activation markers play a role in the initiation, progression and all stages of atherosclerosis. Cell activation and platelet-leukocyte interactions result in the production of a cascade of cytokines, chemokines, adhesion and procoagulant molecules, reactive oxygen species and other proinflammatory molecules that contribute to the disease.

Activated platelets release different cytokines, chemokines and other mediators, including P-selectin, CD40L, and RANTES, which triggers monocyte recruitment and adherence to activated endothelium. RANTES-induced monocyte adherence is mediated by P-selectin. The release of platelet CD40L induces inflammatory responses in endothelium and the release of IL8 and MCP-1, the major chemoattractants for leukocytes. Platelet-monocyte aggregates were found to support monocyte adhesion to endothelium of the vessel wall and these interactions are mainly mediated by interaction between P-selectin and leukocyte PSGL-1. Activated platelets release MMP-2, and interaction between platelets and endothelial cells and monocytes results in the synthesis and release of MMP-9. MMPs are widely expressed in monocytes/macrophages, endothelial cells and smooth muscle cells. Activation of peripheral blood monocytes result in upregulation of CD14/TLR-4 and TLR-2 signaling pathways and increased COX-2 synthesis with production of proinflammatory cytokines, chemokines, tissue factor. Recent studies revealed that TLR-2 and TLR-4 ligation induce secretion of MMP-9 in monocytes. Therefore, production of MMPs after TLR activation and signaling indicate their important role in the development of atherosclerotic disease. In addition, leukocyte MPO can activate MMPs and destabilize atherosclerotic plaques.

We propose to analyze interactions between flow cytometry profiles of platelet and monocyte markers with circulating levels of RANTES, TIMP-1, MMPs.

5. **Main Hypothesis/Study Questions:**

Primary hypothesis:
Cellular markers of platelet and monocyte activation are associated with circulating levels of RANTES and specific MMPs, such as MMP-2 and MMP-9 or the ratio of specific MMPs to TIMP-1, such as MMP-2/TIMP-1 and MMP-9/TIMP-1.

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).**
Analysis of the association between RANTES or specific MMPs, such as MMP-2 and MMP-9 or the ratio of specific MMPs to TIMP-1, such as MMP-2/TIMP-1 and MMP-9/TIMP-1 and cellular markers of platelet or monocyte activation will be done by linear regression of the former variables on each of the flow cytometry variables (one at a time), adjusting for age, sex, and race, accounting for the sampling design using weighting and appropriate software to get correct variances. Next similar models will be run with the additional covariates listed.

Exclusions: missing covariates, any cell and plasma analyte that proved not to be reliable.

Independent variables: All flow cytometry markers and plasma RANTES and MMPs and TIMP-1.

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  _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

   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

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.