1.a. Full Title: Circulating blood platelet-leukocyte aggregates and leukocyte PSGL-1, and carotid artery atherosclerosis (ARIC CAR MRI Study)

b. Abbreviated Title (Length 26 characters):
Platelet-leukocyte aggregates

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

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]

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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 circulating blood platelet-leukocyte aggregates and carotid artery plaque presence/progression/wall thickness. 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 platelet-leukocyte 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.

Activated platelets can bind and form aggregates with leukocytes, and numbers of these aggregates are shown to be increased in patients with CAD. Platelets bind via P-selectin (CD62P) expressed on the surface of activated platelets to the leukocyte receptor, P-selectin glycoprotein ligand-1 (PSGL-1). It has been suggested that platelet-leukocyte aggregates may be more sensitive marker of platelet activation than the surface P-selectin and the measure of platelet-leukocyte aggregates may be a better reflection of plaque instability and ongoing vascular thrombosis and inflammation. Platelet-monocyte aggregates (PMA) were found to support monocyte adhesion to endothelium of the vessel wall and that these interactions are mainly mediated by P-selectin and PSGL-1.

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.

In this study, we measured platelet-monocyte, platelet-granulocyte, and platelet-lymphocyte aggregate levels in the peripheral blood of the ARIC CAR MRI participants. The monocyte-platelet aggregates were identified by CD41 positivity of CD14 labeled monocytes. Blood platelet-granulocytes and platelet-lymphocyte aggregates were identified by their characteristic light scatter properties and positivity for the CD41.

5. **Main Hypothesis/Study Questions:**
a) Peripheral blood platelet-monocyte aggregates reflect the inflammatory alterations of the atherosclerotic plaques.
b) Patients with increased wall volume have increased level of PMA in the circulating blood.
c) Blood PMA levels are independently associated with increased wall volume.
d) Blood PMA level may serve as a novel marker to identify the individuals at increased risk of plaque vulnerability.
e) Leukocyte PSGL-1 expression is altered in advanced carotid artery atherosclerosis/large wall volume.

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).

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

Independent variables: CD14 (MFI), CD41 (%), CD162 (MFI). The proportion of CD14(+) monocytes co-expressing CD41. We will also analyze the level of PSGL-1 (mean or median fluorescence intensity) expressed by free monocytes, and by platelet-monocyte aggregates, respectively.

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  _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

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.