1.a. Full Title: Platelet activation markers and carotid artery plaque presence/progression (ARIC CAR MRI Study)

b. Abbreviated Title (Length 26 characters): Platelets and MRI

2. Writing Group:
   Writing group members:
   Nena Matijevic, Kenneth Wu, Shuyu Yang, 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 blood platelet surface expression of fibrinogen receptor GPIIb/IIIa, platelet activation status expressed by platelet surface P-selectin, platelet surface CD40L, and platelet-platelet aggregates (PPA), and carotid artery plaque presence/progression/wall thickness.

Platelet activation and aggregation are central to the pathophysiology of arterial thrombosis. 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, an endothelial cells. The platelet-leukocyte cross-talk involves a wide range of mediators such as chemokines, adhesion molecules, reactive oxygen species, and cytokines. These cell-cell interactions are mediated by cells surface receptors.

Platelet receptors mediate multiple platelet-vessel wall and platelet-platelet interactions that result in the formation of platelet aggregates. The most abundant receptor on the platelet surface is fibrinogen receptor glycoprotein (GP) IIb/IIIa (integrin apha IIb beta3) (CD41/CD61) and its activation leads to platelet aggregation. Platelets also contain an internal pool of GP IIb/IIIa receptors which externalizes to the platelet surface during the early phase of platelet activation. Activated platelets are characterized by enhanced expression of the GPIIb/IIIa complex. Polymorphism in the IIIa gene Leu33Pro (PLA1/A2) was widely analyzed in relation to the CHD risk suggesting that A2 allele is prothrombotic. In the ARIC study PLA2 allele (less than 2% frequency) was not associated with increased CHD risk. Monoclonal antibody to the GPIIIa (clone SZ21) used for flow cytometry measurements is specific for the PLA1 form of GPIIIa. The expression level of the CD61 on platelet surface should reflect the density of PLA1 expression. In the current study we want to correlate the platelet GP IIb and GPIIIa expression levels with atherosclerosis presence/progression.

P-selectin (CD62P antigen) is a member of the selectin family. It is normally stored in alpha-granules of platelets and is translocated rapidly to the cell surface upon platelet activation. P-selectin mediates rolling platelets and leukocytes on activated endothelial cells as well as interactions of platelets with leukocytes. Platelet P-selectin interacts with P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes to form platelet-leukocyte aggregates. P-selectin is also involved in platelet-platelet interactions, which is major factor in arterial thrombosis. Multiple studies showed increased plasma levels of P-selectin, and other platelet activation markers, beta-thromboglobulin, platelet factor 4, soluble CD40L in patients with coronary artery disease, acute myocardial infarction, stroke, peripheral artery disease, and cerebral vascular disease. Plasma level of soluble P-
selectin represents both platelet and endothelial cell released P-selectin. Platelet surface expression of P-selectin is a direct measure of platelet activation status.

CD40L (CD154), a member of tumor necrosis factor family, exist in soluble and membrane bound forms and is detected on activated T-cells, endothelial cells, smooth muscle cells, and macrophages. CD40L is not expressed on the resting platelet surface. Upon activation, platelets translocate preformed intraplatelet stores of CD40L to the platelet surface. Activated platelets also release large amounts of soluble CD40L. Platelet surface CD40L interacts with endothelial cells and monocytes to induce a thromboinflammatory reaction. CD40L and its receptor CD40 are expressed on a wide range of atheroma-associated cells and their ligation induces expression of cytokines, chemokines, adhesion molecules, matrix metalloproteinases and tissue factors. In clinical studies, patients with acute coronary syndromes express higher platelet levels of CD40L and elevated serum sCD40L.

In this study, we measured the surface expression of platelet membrane glycoproteins IIb (CD41) and IIIa (CD61), platelet surface expression of the P-selectin (CD62P) and CD40L (CD154), as well as platelet-platelet aggregates (PPA).

5. Main Hypothesis/Study Questions:
   a) Peripheral blood platelet surface fibrinogen receptors and activation markers reflect the inflammatory alterations of the atherosclerotic plaques.
   b) Patients with increased wall volume have increased level of platelet receptors GP IIb and GPIIIa.
   c) Patients with increased wall volume have increased level of platelet activation markers P-selectin and CD40L;
   d) Platelet P-selectin and CD40L are independently associated with increased wall volume in atherosclerotic patients.
   e) Participants with large wall volume have elevated levels of the circulating platelet-platelet aggregates.

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).

Platelet membrane CD41 level (mean or median fluorescence intensity), CD61 mean or median fluorescence intensity), CD154 (CD40L) (the percentage of CD41 positive platelets expressing CD40L), CD62P (P-selectin) (the percentage of CD61 positive platelets expressing CD62P and the level of expression measured as a mean or median fluorescence intensity), platelet-platelet aggregates (PPA) (% of CD61 positive events with high forward scatter characteristics). The platelet populations to be evaluated for the expression levels of the antigens include single platelets and platelet-platelet aggregates.
Exclusions: missing covariates, any cell marker that proved not to be reliable.

Independent variables: CD41 (MFI), CD61 (MFI), CD62P (% MFI), CD154 (%), PPA (%).

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.csec.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.