1.a. Full Title: Longitudinal predictors of platelet activation: The Atherosclerosis Risk in Communities (ARIC) Carotid MRI Study

b. Abbreviated Title (Length 26 characters): Cumulative exposure and platelets

2. Writing Group:
Keri L. Monda
Christy L. Avery
Anna Kucharska-Newton
Lloyd Chambless
Nena Aleksic
Aaron Folsom

Other investigators welcome

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

First author:
Address: 137 E. Franklin St., Ste. 32
        CB #8050
        Chapel Hill, NC 27514
Phone: 919.966.8491 Fax: 919.966.9800
E-mail: monda@unc.edu

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).
Name: Kari North
Address: 137 E. Franklin St., Ste. 32
        CB #8050
        Chapel Hill, NC 27514
Phone: 919.966.2148 Fax: 919.966.9800
E-mail: kari_north@unc.edu

3. Timeline: Analyses will begin immediately upon approval. We expect data analysis to be complete by spring 2009 with resulting manuscripts complete by summer 2009.
4. **Rationale:**
Platelets play an essential role in the development of atherosclerosis and subsequent coronary heart disease (Weber 2005; von Hundelshausen and Weber 2007). Platelet activation occurs as a result of exposure to inflamed endothelial cells or following injury to the blood vessel wall and resulting from it exposure to the sub-endothelial matrix (May, Seizer et al. 2008). Platelet activation manifests through increased expression of receptors which allow platelets to bind to the extracellular matrix and to each other (Davi and Patrono 2007). Activated platelets stimulate plaque growth (Dixon, Tolley et al. 2006) and secrete inflammatory mediators (Henn, Slupsky et al. 1998) contributing in that way to a pro-inflammatory microenvironment conducive to progression of atherosclerosis. Association of traditional cardiovascular risk factors with platelet activation has been established through cross-sectional studies (Davi, Catalano et al. 1990; Davi, Gresele et al. 1997; Minuz, Patrignani et al. 2002). Platelet activation is often considered an acute stress reaction, yet the association of platelets with inflammatory stimuli would suggest that long-term exposure to factors such as obesity, hyperlipidemia, alterations in glucose metabolism, and an increase in shear stress would create a milieu conducive to ongoing platelet stimulation. Activation of platelets could therefore reflect an acute phase reaction occurring in the context of a chronic inflammatory condition. Longitudinal studies are needed to establish the possibility of such an association.

In this study, we propose to examine the association of cumulative exposure to traditional cardiovascular risk factors with measures of platelet activation: mean platelet volume, density of selected platelet surface markers and levels of platelet-monocyte aggregates. It is to be based on data from the CarMRI study, an ancillary study of the Atherosclerosis Risk in Communities (ARIC) Study that was conducted in 2004-2005, at study calendar year 18 (also referred to as Visit 5). CarMRI investigators obtained contrast enhanced MRI image data of the carotid artery of approximately 2000 individuals (1200 with high carotid artery wall thickness and a weighted sample of 800 with normal carotid artery wall thickness) as well as performed whole blood flow cytometry analysis of platelet and monocyte surface markers. These unique data, combined with data collected over the previous four visits, is exceptionally well-suited to examine associations of cumulative exposure to traditional cardiovascular disease risk factors with markers of platelet activation.

5. **Main Hypothesis/Study Questions:**
1. Cumulative exposure to traditional risk factors measured from 1987 through 2004 is associated with increased density of selected platelet surface markers: CD41, CD61, CD62P
2. Cumulative exposure to traditional risk factors measured since 1987 through 2004 is associated with increased levels of platelet-monocyte 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).

Outcome variables
Of the identified platelet surface markers, CD41 (platelet glycoprotein GPIIb), CD61 (platelet glycoprotein GPIIIa), CD62P (P-selectin), and CD154 (CD40L) have been shown to have good between-visit and within-visit repeatability in the CarMRI flow cytometry study (Catellier, Aleksic et al. 2008), therefore densities of those markers will constitute outcome variables in this study.

Exposure variables
The traditional cardiovascular disease risk factors we propose to examine as cumulative exposure measures are as follows:

<table>
<thead>
<tr>
<th>Anthropometrics</th>
<th>BMI, waist circumference, waist-hip ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lipids/lipoproteins</td>
<td>Total, LDL, and HDL cholesterol, triglycerides</td>
</tr>
<tr>
<td>Diabetic indicators</td>
<td>Glucose, insulin, HOMA</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>Systolic and diastolic</td>
</tr>
</tbody>
</table>

Statistical methods
We propose to examine the association between cumulative exposure to traditional risk factors and platelet activation measured as part of the flow cytometry component of the CarMRI study.

We will build on the work of Folsom et al. (Folsom et al., 2008) examining the association of traditional cardiovascular risk factors and platelet activation. We will utilize methods traditionally used to examine longitudinal exposure data, e.g. defining exposure as the mean over time, the sum of exposures over time, and the difference between proximal and distal measures of the exposure. Cumulative exposure variables (CEV) have been developed by Dr. Chambless and defined as the cumulative area of the exposure for the five ARIC visits divided by the total time of follow-up. These trapezoidal measures are interpreted as the average value of the exposure variable over the period of time from Visit 1 to Visit 5.

We propose to extend Dr. Chambless’ efforts by exploring additional cumulative exposure metrics, including the method developed by Cook et al. (Cook, Rosner et al. 2004). Briefly, we will use the multiple exposure measurements of various traditional cardiovascular risk factors calculated over 18 years of follow-up and longitudinal growth curve models to estimate each participant’s area under the curve (AUC), interpreted as the average value of exposure since baseline examination. We will use these AUC estimates to fit linear predictive models for platelet activation (measured at Visit 5). We will evaluate creating growth trajectories restricting analyses to individuals in the CarMRI study as well trajectories using the entire ARIC cohort. Additional longitudinal methods under evaluation include the distributed lags (Pope, 1996) method and categorical growth trajectories (Jones et al., 2001).
We recognize that the age range of ARIC participants spans 20 years. We will examine growth trajectories by age, including stratification by several categories of age at baseline. This approach will allow us to determine how robust the growth trajectories are to different levels of extrapolation.

We will of course be sensitive to exclusion criteria, including the application of CarMRI sampling weights. Potential confounders/effect modifiers (e.g. smoking, race, sex, study center, and other traditional CVD risk factors) of the association of the cumulative exposures and platelet activation will be evaluated using directed acyclic graphs.

While previous analyses using the CEV measures resulted in null findings, there are a number of differences between the methodologies that we feel would warrant reevaluating cumulative exposure metrics. For instance, the model-based method developed by Cook et al is independent of the ages at measurement and is able to extrapolate over the entire age range. Further, we can specify the AUC to be a function of not only time, but of sex, race, and other characteristics deemed important. Similarly, it is possible to allow growth trajectories to be nonlinear by including a quadratic term in the model. Finally, these models can accommodate short-term fluctuations (within-person variability) around an individual’s growth pattern. Other strengths of alternative longitudinal methods include accommodation of unbalanced repeated measurements, use of all available data for an individual, and borrowing information from the entire cohort experience when measurements are missing.

7.a. Will the data be used for non-CVD analysis in this manuscript?  ____ Yes  ___X___ No

b. If Yes, is the author aware that the file ICTDER03 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 ICTDER03 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  ___X___ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = “No use/storage DNA”?  _____ Yes  _____ No

8.c. If yes, is the author aware that the participants with RES_DNA = ‘not for profit’ restriction must be excluded if the data are used by a for profit group?  _____ 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

__X___ 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)?
MS #1206 (Folsom et al.): Association of established risk factors with blood platelet and monocyte cell-markers and cell aggregates (ARIC MRI).

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ______ Yes __X__ 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.

References:


