

ARIC Manuscript Proposal #1347

PC Reviewed: 02/12/08
SC Reviewed: _____

Status: A
Status: ___

Priority: 2
Priority: _

1.a. Full Title: Relationship between circulating levels of RANTES(regulated on activation, normal T-cell expressed, and secreted) and carotid plaque characteristics; the ARIC Carotid MRI Study.

b. Abbreviated Title (Length 26 characters): RANTES and Carotid artery plaque characteristics.

2. Writing Group:

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

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3. Timeline: We plan to analyze the data as soon as approval is obtained. Manuscript will be prepared as soon as analysis is done. We plan to do the analysis, as well as prepare the manuscript for submission within 1 year.

4. Rationale: CCL5 or RANTES (regulated on activation, normal T-cell expressed, and secreted) is a chemokine that is stored in the alpha granules of the platelets [Gear AR, Microcirculation. 2003;10:335-350]. After release from the activated platelets, it can be deposited on endothelium and has been shown to mediate transmigration of monocytes

into the inflamed intima [Charo IF, *Circ Res.*2004;95:858-866]. Data regarding the significance of RANTES in the atherosclerotic disease process, as well as its role in plaque vulnerability remain controversial. In some studies especially those involving patients with acute coronary syndromes, levels of RANTES have been found to be elevated [Nomura S, *Thromb Haemost.* 2003;89:506-512], whereas low levels of RANTES have been shown to be independently predictive of adverse cardiac outcomes in patients with chronic coronary artery disease [Cavusoglo E, *Arterioscl Thromb Vasc Biol.* 2007;27:929-935]. Similarly, the relationship between RANTES levels and carotid atherosclerosis has not been well studied.

Platelets sequester RANTES in the alpha granules and release it during acute phase of inflammation. Since RANTES are known to be very potent chemoattractants for a variety of cell types including T cells, monocytes, and natural killer cells [Von Hundelshausen, *Circulation.* 2001;103:1772-1777), it is possible that high levels of RANTES would lead to a more cellular infiltrate in the plaques. It is known that vulnerable plaques characteristically have a prominent lipid core, thin fibrous caps, and a large number of macrophages [Davies MJ, *British Heart Journal.* 1993;69:377-381]. Higher levels of RANTES in theory would lead to recruitment of more macrophages into the plaque, which in turn could secrete matrix metalloproteinases (MMP) [Galis ZS, *Circulation Research.* 2002;90:251-262], leading to breakdown of the fibrous cap making these plaques unstable or vulnerable.

The aim of the current manuscript proposal is to assess the relationship between plasma levels of RANTES and total carotid plaque volume, as well as the development of high risk carotid plaques in the ARIC cohort measured using carotid MRI.

MRI of the carotid arteries were done in nearly 2000 patients in the ARIC cohort. The ARIC MRI cohort included 1200 participants whose carotid artery wall thickness as measured by carotid B mode ultrasound on visit 3,4 was at least >68 percentile (The IMT cut-offs were 1.35, 1.00, 1.28, and 1.22 mm at Forsyth County, Jackson, Minneapolis suburbs, and Washington County, respectively, representing the 73rd, 69th, 73rd, and 68th percentiles of maximal IMT from Exam 4), and a cohort random sample of nearly 800 participants whose carotid intima-media thickness was <68 percentile. The carotid MRI procedure included measurements of maximal wall thickness, carotid wall volume, luminal area, lipid core volume and maximum area, fibrous cap thickness using gadolinium enhanced MRI on thicker internal carotid artery using a 1.5 T magnet. Plasma samples of all these patients are also available from visit 5. This gives an opportunity to identify the relationship between plasma levels of RANTES and the presence of high risk plaques as described by thick lipid rich core and thin fibrous cap on carotid MRI.

5. Main Hypothesis/Study Questions:

Plasma levels of RANTES are independently associated with total carotid plaque volume as well as high risk plaques on carotid MRI in the ARIC cohort.

Specific study questions:

1. To determine if plasma levels of RANTES are independently associated with carotid atherosclerosis as measured by increased wall thickness, or total wall volume in the carotid MRI study.
2. To determine if plasma RANTES levels are associated with high risk plaque (*i.e. thin fibrous cap with large lipid core) compared to stable plaque, among those participants with plaque (*We will explore the definition of high risk plaque based upon measurements of fibrous cap thickness or volume and lipid core volume or area)
3. To determine if plasma RANTES levels are associated with the inflammatory marker hsCRP and traditional lipid risk factors.

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).

The study design is cross sectional. All individuals enrolled in the ARIC MRI study who have had plasma RANTES levels measured and have good quality MRI will be eligible for this analysis. Of all the carotid MRI performed, a total of 1769 have been reported to be of good quality.

MRI variables will include:

GDSICA-TOTALWALLVOLUME (total wall volume based on 8 slices)

GDSICA-MAXWALLTHICK-MAXCORE (maximum segmental wall thickness of 12 segments at slice with largest lipid contour area)

MEAN-CAP-THICKNESS-2ADJACENT (Mean segmental cap thickness with at the 2 adjacent slices with largest lipid core)

MEAN-MIN-CAP-THICKNESS-2ADJACENT (Mean of the two minimum cap thickness of the 2 adjacent slices with largest lipid core)

LUMENAREA-MAXWALL

VESSELWALLAREA-MAXMEANWALL

GDSICA-MAXCALCIUMAREA (Maximum calcium area at any site)

GDSICA-TOTALLIPIDCOREVOLUME (Total lipid core volume on 8 slices)

GDSICA-MAXLIPIDCOREAREA (Maximum lipid core area of 8 slices)

LIPIDCORE

Plasma RANTES levels will be divided up into quartiles initially (highest and lowest quartiles will be compared for analysis) to assess if there are any differences between highest and lowest quartiles of RANTES levels and the presence or severity of the MRI parameters mentioned above. These analyses would be done to assess if there is any association between quartiles of RANTES levels with either total wall volume, presence of lipid rich core, or the presence of thin fibrous cap on carotid MRI. Subsequently, RANTES levels will be used as continuous variable to see if there is a linear relationship between increasing levels of RANTES and the above mentioned parameters on carotid MRI.

A regression analysis will subsequently be used to see if serum RANTES levels remain independent predictors of increased wall thickness, presence of lipid rich core, and presence of thin fibrous cap, when adjusting for several covariates. Covariates will include age, gender, BMI, diabetes, smoking (never, current, and former), systolic and diastolic blood pressure, total cholesterol, and current drug regimen (lipid lowering, anti-hypertensive, anti-diabetic, anti-inflammatory, aspirin use, plavix use).

All analysis is based on methods appropriate for stratified random sample methods. In particular, all analyses are weighted by the inverse of the sampling fractions in the 8 sampling strata (4 field centers X 2 IMT groups) The association between MRI variables and RANTES will be analyzed by linear regression for continuous MRI variables and logistic regression for categorical MRI variables, with the MRI variables as the dependent variables, adjusted first for Model 1 (basic model):age, sex, and race, and then additionally for other covariates, including Model 2: Model 1 + total cholesterol, HDL-C, and triglycerides, and Model 3: Model 2 + smoking, BMI, blood glucose, blood pressure, use of blood pressure-lowering medication, lipid-lowering medication, aspirin, plavix, anti-arthritis medication, diabetes medications, and CRP. The association between RANTES and fibrous cap thickness will be analyzed both as a continuous variable and categorized as “thin” and “thick”.

For adjustment for standard risk factors, outside of age, sex, and race, the analysis will consider both concurrent (cross-sectional) measures of risk factors as well as cumulative exposure or rate of change of exposure. The cumulative exposures will be determined for continuous variables as the area under the curve of exam-specific values plotted versus exam time, divided by time between first and last exam. This can be interpreted as the estimated mean daily value over the period. For dichotomous risk factors the cumulative indicator is the proportion of time exposed. For the continuous variables we will calculate the rate of change over the period as the person-specific slope from a random coefficients linear model.

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.csc.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.csc.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.