

ARIC Manuscript Proposal # 1503

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1.a. Full Title: Risk factors for subtypes of cerebral infarct-like lesions and white matter hyperintensities detected by magnetic resonance imaging in the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): MRI ILL subtypes

2. Writing Group:

Writing group members (not in order): Daniel C. Bezerra, Josef Coresh, Rebecca F. Gottesman, Kunihiro Matsushita, Thomas H. Mosley Jr., A. Richey Sharrett, Moyses Szklo, Elizabeth Selvin

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

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3. Timeline: Completion in 18 months

4. Rationale:

As recently published by Wardlaw: “Lacunes are small (3 to 15mm) cerebrospinal fluid filled cavities located most often in the basal ganglia or white matter, representing

subcortical ischemic infarcts resulting from the occlusion of a small perforating artery deep in the brain or the brain stem, that are frequently found on imaging in older people” [1-3]. According to the same author, “lacunar infarcts” found by cerebral imaging should be differentiated from “lacunar stroke”, a term that ought to be used only in the presence of lacunar clinical stroke symptoms in addition to an infarct on brain imaging. “Lacunar infarct” refers to an ischemic lesion on brain-imaging which may or may not be associated with a clinically recognized “lacunar stroke”.

The pathogenesis of lacunes has not been fully clarified, and some authors proposed that there may be different lacunar entities [4-10]. At autopsy, Fisher described two vascular pathologies underlying lacunar infarcts: (1) lipohyalinosis (or arteriolosclerosis), observed in hypertensive patients, usually with one or more smaller lacunar infarcts and no cerebral symptoms, often associated with white matter hyperintensities (or lesions, WML, or leukoaraiosis); and (2) microatheromatous disease, usually linked to single, larger, often symptomatic lacunes [5;7-10]. Knowledge of possible differences in risk factor pattern between these two types is quite limited.

There are some observational studies supporting the hypothesis of two different vascular pathologies [6;11-15]. For instance, in a single center observational study involving patients admitted for their first clinical lacunar syndrome [6], the authors found that there were different factors associated with the presence of multiple vs. single lacunes on brain magnetic resonance imaging (MRI) at hospitalization. After adjustment for age and other potential confounders, diabetes mellitus (OR 2.43; 95%CI 1.09-5.4) and leukoaraiosis by MRI (OR 3.58; 95%CI 1.77-7.51) were associated with multiple but not single lacunar infarcts. Furthermore, during a median follow-up of 12 months, symptomatic patients with multiple lesions had a higher risk of recurrence (24.3% vs 7.7%) and poorer functional outcome (OR 5.4; 95%CI 1.25-23.9).

In the Leukoaraiosis and Disability in the Elderly Study (LADIS) [14], incident lacunes in the subcortical white matter occurred more often in persons who had preexisting WML, and they were accompanied by new and expanded WML, compared to incident lacunes located in the basal ganglia. The lacunes located in the subcortical white matter are likely to be associated with Fisher’s “lipohyalinosis” (arteriolosclerosis), whereas the basal ganglia infarcts, located closer to the origin of the lenticulostriate arterioles, are more likely to be associated with Fisher’s “microatheromatous disease”.

Risk factors for infarct-like lesions – broad objectives

The overall objective of this proposal focuses on risk factors which are known to be associated with microvascular disease (arteriolosclerosis), in particular, markers of impaired glucose metabolism (such as diabetes, fasting glucose, insulin and HbA1c levels). We will examine the association of these and other vascular risk factors with very specifically defined cerebral small vessel disease assessed by MRI in ARIC. The small vessel disease of interest is defined by those lacunes (or “infarct-like lesions” in ARIC MRI data) which are believed to be associated with arteriolosclerosis, as defined more specifically in Section 5 below.

The strong associations of diabetes with retinopathy, renal disease and other manifestations of microvascular disease are well known. Furthermore, treatment of

hyperglycemia is more consistently linked to decreased microvascular disease than to reduction in the incidence of atherosclerotic complications [23].

Diabetes mellitus is a well known risk factor for all subtypes of stroke, and although in the Atherosclerosis Risk in Communities (ARIC) its association was stronger for clinically recognized lacunar than for nonlacunar stroke [17], in a recent systematic review [18], diabetes seemed to be equally associated with clinically recognized small and large artery disease. However, it is to be noted that clinically recognized lacunar strokes are the larger lacunar strokes, and thus likely to be of Fisher's microatheromatous type, more similar to large artery disease, than the arteriolosclerosis-associated type of lacunar infarcts. Studies of asymptomatic populations have not consistently supported the association of MRI-detected lacunar infarcts with diabetes. Some showed positive associations [15;19;20] as we hypothesize here, but others showed null associations [21;22]. However, none of the population-based studies reported on differences between the subtypes of lacunar infarction we describe here, specifically the distinction between lacunes associated with arteriolosclerosis vs. those associated with microatheromata.

Our specific expectation is that in ARIC, the presence of diabetes and other markers of impaired glucose metabolism will be more strongly associated with MRI-detected infarct-like lesions (ILL) associated with cerebral arteriolosclerosis, as defined below, than with other ILL. This expectation is supported by the fact that many studies suggest that either diabetes [24;25] or glycated hemoglobin [26] is associated with the presence of WML. This association too is supported by most of the larger population-based imaging studies [15;27;28] but not all of them [29;30]. Since WML are associated more with arteriolosclerotic than with other ILLs, concurrence with greater WML grade will be an important marker for arteriolar ILL in this study.

Thus, we propose to study the association between cardiovascular risk factors, particularly markers of impaired glucose metabolism such as diabetes, insulin (measured in visit 1 only), glucose and HbA1c levels (measured in visit 2 only), and the presence, location and size of MRI-detected ILL and their associated WML. Although the relationship between the presence of diabetes mellitus and stroke has already been studied in the ARIC, emerging data indicate that higher HbA1c levels are also linked to stroke [31] and coronary heart disease [32].

5. Main Hypothesis/Study Questions:

As reported by Fisher, we hypothesize that WML grades will be higher in

- 1.1. Persons who have ILL than in those who do not have ILL
 - 1.2. Persons who have small ILL (defined as those with ≤ 6 mm maximum diameter) than in those with large ILLs only
 - 1.3. Persons whose ILLs are located in the deep WM (as classified in the ARIC database), than in persons whose only ILLs are located elsewhere, and
 - 1.4 Persons with multiple ILL lesions than in persons with only a single ILL.
2. These hypotheses, if supported, would reinforce the concept of a specific arteriolar type of lacunar entities (or infarct-like lesions) and suggest the importance of

examining the associations of specific markers of that arteriolar type of lacunes with vascular risk factors. Since markers of impaired glucose metabolism appear to show particularly strong associations with microvascular disease, we hypothesize that the associations described in 2.1 to 2.4 below will be strong:

- 2.1. Among persons with MRI imaging, markers of glucose metabolism (e.g. elevated fasting glucose levels, elevated fasting insulin levels, elevated HbA1c and diabetes) will be associated with a higher WML grade.
- 2.2. Among persons with MRI imaging, markers of glucose metabolism will be positively associated with the presence of small ILL (defined as ≤ 6 mm in maximum diameter).
- 2.3. Among persons with MRI imaging, markers of glucose metabolism will be positively associated with ILL location in the deep WM as defined in the ARIC database.
- 2.4. Among persons with non-cortical ILLs, markers of glucose metabolism will be positively associated with the presence of more than one ILL.
- 2.5. We will examine the association other cardiovascular risk factors with the endpoints defined above (2.1-2.4) without a priori expectations regarding the strength of association.

3. A combination of the markers of cerebral arteriolar disease may index the presence and severity of cerebral arteriolar disease more accurately than the individual markers alone. Therefore we will develop a crude “arteriolar index” using each marker: higher grade WML (hypothesis 2.1); presence of smaller ILLs (2.2); presence of deep ILLs (2.3); and the presence of more than one non-cortical ILL (2.4). The score would be comprised of those 4 factors, scored 0,1 for each and range in value from 0-4. In order to examine the differential association risk factors with lacunes of the arteriolar type we would limit the analysis to persons with ILL on MRI imaging hypothesize that, among these persons:

- 3.1 Markers of glucose metabolism would be positively associated with this “arteriolar index”.
- 3.2 We will examine the association other cardiovascular risk factors with the “arteriolar index” defined above without expecting a priori a positive association.

For example, hypertension, which is associated with all types of infarcts, may show no association with arteriolar index among persons with ILL.

All item 2 and item 3 hypotheses relate to unadjusted associations. However, as indicated in Statistical Analysis below, models adjusted only for demographics will be supplemented by models including the major vascular 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 population of this study will consist of those who participated in the cerebral MRI investigation at two sites in the ARIC study [33-36]. Other analyses, as specified, will be limited to those participants who were seen to have ILL.

The ARIC cohort was selected as a probability sample of 15,792 men and women between the ages of 45 and 64 years at four study centers in the United States. Three of these four study centers enrolled an ethnically diverse population; the fourth cohort was sampled from black persons who were residents of Jackson, MS. Details of sampling, study design, and cohort examination procedures have been published [37]. The baseline examination of the ARIC cohort was conducted from 1987 through 1989. Every 3 years after the baseline examination, all participants were invited to a follow-up clinical examination.

MRI variables:

MRI data are available from visit 3 (1993 through 1995), on the sample of cohort members who were 55 years old and older (n=2825) at the Forsyth and Jackson study sites was screened for eligibility and attended the cerebral MRI examination. The final sample size for this study was 1890 (1127 women; 763 men; 964 white; 926 black) [33].

Infarct-like lesions (ILL), which we will study in the proposal described here, were defined as focal, non-mass lesions having arterial vascular distribution and being hyperintense to gray matter on both spin-density and T2-weighted images. The intensity of the lesions on the T1-weighted images relative to normal gray matter was recorded. To be considered an ILL in cerebral white matter and the brain stem, lesions were required to be hypointense on T1-weighted images, similar to cerebrospinal fluid [33;38]. The dimensions of the lesions were measured carefully using an electronic cursor; the maximal right-to-left and anterior-to-posterior dimension of each lesion was recorded. The superior-to-inferior dimension was reported by the number of axial sections on which the lesion appeared. Lesions with a diameter of less than 3 mm could not be measured accurately because of pixel resolution, and these will not be considered in the proposed analysis. They were recorded simply as “less than 3 mm”. For anatomic localization, lesions were assigned to one or more of 23 anatomic regions defined by gross anatomic and vascular characteristics.

Spin-density images (repetition time, 3000 milliseconds; echo time, 30 seconds) were used to estimate the overall volume of periventricular and subcortical white matter signal abnormality. These were coded on a scale from 0 to 9, based on “pattern matching” of a scan to a set of reference standards, as described [27;35]. The reference standards are: no white matter signal abnormalities (grade 0); discontinuous periventricular rim or minimal “dots” of subcortical white matter (grade 1); thin continuous periventricular rim or few patches of subcortical white matter lesions (WML) (grade 2); thicker continuous periventricular rim with scattered patches of subcortical WMLs (grade 3); thicker, shaggier periventricular rim with mild subcortical WMLs—may have minimal confluent periventricular lesions (grade 4); mild periventricular confluence surrounding the frontal and occipital horns (grade 5); moderate periventricular confluence surrounding the frontal and occipital horns (grade 6); periventricular confluence with moderate involvement of the centrum semiovale (grade 7);

periventricular confluence involving most of the centrum semiovale (grade 8); and all white matter involved (grade 9). Inter- and intra-reader intraclass correlations were 0.68 and 0.71, respectively [27].

In the ARIC ILL prevalence study [33] there were 435 ILLs (45 cerebral, 148 in the deep white matter, and 195 in the thalamus and basal ganglia, and 47 in the posterior fossa). In this same study, there were 69 ILLs over 16 mm in size as compared to 296 with maximum diameter of 3 to 15mm in diameter.

Covariates

The ARIC examination consisted of collection of a blood sample [39-41] and a standardized interview [42]. Cigarette smoking was ascertained from interview. Blood pressure was determined using a random-zero sphygmomanometer and the mean of the last 2 measurements was calculated. Derived variables will be used for hypertension, diabetes and carotid thickness. Hypertension was defined as systolic blood pressure of 140 mm Hg or greater, diastolic blood pressure of 90 mm Hg or greater, or use of antihypertensive medication during the previous 2 weeks. Diabetes mellitus was defined as a fasting glucose level of at least 126 mg/dL (7.0 mmol/L), a nonfasting glucose level of at least 200 mg/dL (11.1 mmol/L), or a self-reported history of physician-diagnosed diabetes or treatment for diabetes. Average carotid intima-media wall thickness (IMT) was measured using B-mode ultrasonograms [42]. Assays of total cholesterol, high-density lipoprotein cholesterol, and glucose levels are described elsewhere [42]. Frozen whole-blood samples obtained at the second ARIC visit were thawed and assayed for HbA1c with a Tosoh HPLC instrument (Tosoh Aic 2.2 Plus HPLC, Tosoh Medics, Foster City, CA, USA) [43] and insulin levels were measured only at visit 1.

Statistical Analysis (sections correspond to hypothesis numbers above)

1.1, 1.2 Since the size of the lesions is not normally distributed, we will assess the crude association between the WML grade and ILL size (in mm) by Spearman correlations. In addition, WML will be dichotomized as grade 3 or higher or lower (corresponding to the 90th percentile) [34;35] and we will use a Wilcoxon Rank Sum Test to evaluate whether the presence of the ILLs (hypothesis 1.1) or their sizes (1.2) differ between these two categories.

1.3, 1.4 The associations between ILL location in the deep cerebral WM (n=86) and WML grades and between the number non-cortical ILL lesions (n=390) and WML grades will be assessed initially using cross-tabulations.

We will use logistic regression models with the dichotomized WML grade (as \geq 3 vs. <3) a dependent variable, in order to quantify the association between each of these variables (presence, location and number of ILL) and the WML grade, in models adjusted for age, sex and ethnicity.

In addition, the WML grades will also be studied using polytomous regression models to determine a possible graded effect treating WML grade as a nominal outcome (i.e. grades 0-1 reference, grade 2, grade 3 and grades 4-9)[34] and possibly a Generalized Additive Model (GAM) using Z score for WML [44].

Item 2 hypotheses will be examined as follows.

- 2.1. The association of risk factors with the WML grade will be assessed initially by Spearman correlations.
- 2.2. The association of risk factors with ILL size will be assessed initially by Spearman correlations and by examining mean values of the risk factors within ILL size strata dichotomized as \leq or $>$ 6mm (t-test).
- 2.3. The association of risk factors with ILL location will be assessed initially by examining mean values of the risk factors in two location categories: deep WM vs. other (t-test).
- 2.4. Risk factors will be associated with the number of non-cortical ILLs either continuously (Spearman correlations) or when dichotomized by one vs. more than one ILL (t-test of difference in means)
- 2.5. Each marker of arteriolar disease (smaller size of the ILL, number of ILL lesions, WML grade, ILL location), considered as a dependent variable, will be evaluated by a logistic or continuous multivariate model (as appropriate adjusting for demographics (model 1) and vascular risk factors (model 2, including hypertension, smoking, cholesterol, and a marker of impaired glucose).

Item 3 hypotheses will be examined as follows:

- 3.1 Associations of markers of impaired glucose metabolism with the “arteriolar index” will be assessed initially by Spearman correlations and then by multivariate regression (with the arteriolar index the dependent variable considered as an ordinal or a continuous or log-transformed continuous variable).
- 3.2 Association of other cardiovascular risk factors with the “arteriolar index” will be assessed similarly. These hypotheses will be evaluated in both simple and more complete models as described in 2.5 above).

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 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
 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.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)?

Ms# 1387 Gottesman. trends in blood pressure and cerebral white matter lesions in a biethnic sample

Publications:

1. Bryan RN, Cai J, Burke G, Hutchinson RG, Liao D, Toole JF, Dagher AP, Cooper L. Prevalence and anatomic characteristics of infarct-like lesions on MR images of middle-aged adults: the atherosclerosis risk in communities study. *AJNR Am J Neuroradiol* 1999;20:1273-1280.
2. Liao D, Cooper L, Cai J, Toole JF, Bryan NR, Hutchinson RG, Tyroler HA. Presence and severity of cerebral white matter lesions and hypertension, its treatment, and its control: the ARIC Study. *Stroke* 1996;27:2262-2270.
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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)* 2003.05 _____
2006.15 _____)

*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.

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