1.a. **Full Title**: Associations between lacune subtypes and retinal microvascular disease, renal markers and intima-media thickness in ARIC

b. **Abbreviated Title (Length 26 characters)**: Retina IMT ILL subtypes

2. **Writing Group**:
   Writing group members:(not in order):
   Daniel C Bezerra, A. Richey Sharrett, Marilia Sá Carvalho, Barbara Klein, Dean Shibata, Josef Coresh, Thomas Mosley, Rebecca Gottesman

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**:18 months

4. **Rationale**:
   Lacunes are small (1-20mm) cerebrospinal fluid filled cavities caused by the occlusion of small arteries in the brain. When symptomatic, they may be interpreted as lacunar strokes[1,2].
The pathogenesis of lacunes has not been fully clarified, and there is pathological and clinical evidence that there may be distinct types of lacunar entities[3-5]. At autopsy, Fisher described two main vascular culprit lesions: (1) lipohyalinosis, observed in hypertensive patients, usually with one or more small lacunar infarcts (up to 7mm), located in the deep white matter and thalamus, being rarely symptomatic; and (2) microatheromata, usually linked to larger (5-20mm), more often symptomatic lacunes[2,3] present in the basal ganglia and brainstem. This pathological distinction remains unchallenged. Although contemporary authors describe several types of small vessel disease associated with lacunes[2,6,7], we will, for convenience, consistently use only the term “lipohyalinosis” in this proposal to refer to the type of arteriolar lesion underlying lesions ≤ 7 mm, without intending to support any of the term’s more specific pathogenic implications.

There are a few observational studies supporting the hypothesis of two different vascular pathologies[5,8-11]. For instance, in a single center observational study involving patients admitted for their first clinical lacunar syndrome[5], 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 leukoaraisosis by MRI (OR 3.58; 95%CI 1.77-7.51) were associated with multiple but not single lacunar infarcts.

**Risk factors for subtypes of infarct-like lesions: broad objectives**

The overall objective of this proposal is a follow up of an earlier ARIC paper (ARIC ms# 1503) and focuses on studying further risk factors for subtypes of lacunes according to their presumed pathology by using brain MRI data from ARIC.

Since lacunar infarction involves small vessels in the brain, one would expect that risk factors classically associated with microvascular disease, such as diabetes mellitus, would be more strongly associated with this stroke subtype as compared to others. Nevertheless, in a recent meta-analysis diabetes seemed to be equally linked with lacunar and other subtypes of stroke[12]. Also, intima-media thickness (IMT) was similar in patients with lacunar strokes compared with those with large artery disease, despite the expectation that it would be greater in the latter[13]. 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, and therefore more similar to large artery disease, than the lipohyalinosis-associated type of lacunar infarcts.

Thus, studies of largely asymptomatic populations, as the ARIC, would be more supportive of this hypothesis. Nevertheless, even in this situation, lacunar lesions detected by MRI were not consistently associated with diabetes[14]. Possible explanations to this observation are: the fact that most studies tend to ignore the pathological subtypes of lacunes; the inclusion of lesions of “nonlacunar” etiology[15], such as those extending to the cortex; and the frequent exclusion of lesions <3mm, assuming that they represent enlarged perivascular spaces[14,15], although recent evidence indicate that they may be a marker of cerebral small vessel disease[7,16,17].

In the most specific test to-date of the hypothesis of distinct lacune types, some of the authors involved in this proposal (Bezerra, Sharrett, Carvalho, Shibata, Coresh, Mosley and Gottesman) recently evaluated the hypothesis that lacunes classified by their
presumed underlying pathology would be associated with different risk factors, specifically, that diabetes and HbA1c would be preferentially associated with the smaller lesions (ARIC # 1503; Risk factors for subtypes of cerebral infarct-like lesions detected by magnetic resonance imaging in the ARIC Study; submitted). The authors examined ARIC's cerebral MRI data[18] and divided non-cortical infarct-like lesions (ILLs) into two strata: those ≤7mm (more likely to be of lipohyalinotic origin) and 8-20mm (probably due to microatheroma). The number of each type of lesion was used as a dependent variable in a Poisson model and when ILLs ≤7 mm were used as a response variable, lesions in the 8-20mm range were counted as “0” and vice-versa. Age (prevalence rates - PR 1.11 per year; 95%CI 1.08-1.14), black ethnicity (PR 1.66; 95%CI 1.27-2.16), hypertension (PR 2.12; 95%CI 1.61-2.79), diabetes (PR 1.42; 95%CI 1.08-1.87) and ever-smoking (PR 1.34; 95%CI 1.04-1.74) were significantly associated with the number of ILLs ≤7 mm. On the other hand, ILLs 8-20mm were associated with age (PR 1.14 per year; 95%CI 1.09-1.20), hypertension (PR 1.79; 95%CI 1.14-2.83), ever-smoking (PR 2.66; 95%CI 1.63 - 4.34) and LDLc (per SD mg/dL; PR 1.27 per SD; 95%CI 1.06-1.52), but not diabetes. Associations for HbA1c, as well as those for diabetes, also supported their prior hypothesis. Furthermore, even tiny ILLs <3mm had risk factor associations similar to infarcts in the 3-7 mm size class, supporting the idea that they could be a marker of cerebral small vessel disease[7,16,17].

5. Main Hypothesis/Study Questions:

We hypothesize that lacunes classified by their size (and presumed underlying pathology, lipohyalinosis vs. microatheroma) are associated with different indicators of macro- and micro-vascular disease. Thus, we hypothesize that the smaller (lipohyalinotic) lesions will be associated with other markers of microvascular disease such as retinal vascular signs[19-21], and impaired renal function[22,23]. On the other hand, lesions due to microatheroma should be more likely associated with risk factors for large vessel atherosclerosis, i.e. carotid IMT[24].

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 methodological limitations or challenges if present).

This study will include the subjects who completed the MRI and retinal examinations at ARIC visit 3 and at least one carotid ultrasound scan at visit 1 or 2.

MRI variables:

Infarct-like lesions (ILL) were defined as focal lesions hyperintense to gray matter on both proton-density and T2-weighted images. To be considered an ILL in cerebral white matter, lesions were required to be hypointense on T1-weighted images, similar to cerebrospinal fluid[18]. The dimensions of ILLs 3mm or greater were recorded using an electronic cursor, recording maximal right-to-left and anterior-to-posterior dimension of each lesion. Lesions with right-to-left or anterior-to-posterior measurements of less than 3 mm were recorded simply as “less than 3 mm”. Lesions <3mm were
Lesions were assigned to one or more of 23 anatomic regions defined by gross anatomic and vascular characteristics. Since we are limiting our study to lacunar disease, we will only consider noncortical lesions of less or equal to 20 mm in maximum dimension located in the caudate, lenticular nucleus, internal capsule, thalamus, brainstem, deep cerebellar white matter, centrum semiovale or corona radiata.

**Covariates**

In ARIC ms#1503, of the 1930 participants who underwent MRI, 1827 were not missing variables of interest and made up our final study sample. We expect a similar number of subjects in the present study, subject only to further exclusions based on missingness of retinal or ultrasound variables. The risk factors used as covariates will be the classic CHD risk factors used in ms#1503 (see below).

**Statistical Analysis**

As in the corresponding ARIC article, non-cortical ILLs will be divided into two strata: those ≤7mm (more likely to be of lipohyalinotic origin) and 8-20mm (probably due to microatheroma). The primary analysis will involve the number of each type of lacunar lesion as a dependent variable in a generalized linear model assuming the Poisson distribution. When ILLs ≤7mm are used as an outcome variable, lesions in the 8-20mm range will be counted as “0” and vice-versa. The models will include a priori these selected variables: age, gender, race, smoking, diabetes and markers of impaired glucose metabolism, IMT, retinal lesions and indicators of impaired kidney function (i.e.: serum creatinine, estimated glomerular filtration rate and cystatin-C), using separate models for each lesion size class. Retinal variables for consideration include arteriolar width (CRAE) and venular width (CRVE) (considered as continuous variables), and the following categorical variables: focal arteriolar narrowing (15% prevalence), AV nicking (14% prevalence) and retinopathy (7% prevalence) and the separate retinopathy signs. Overall mean IMT and ankle-brachial index (ABI) from visit 1 will be used as markers of large artery atherosclerosis. Associations with retinal variables will be considered in diabetic and non-diabetic participants separately. The models will be later examined for their goodness-of-fit and the presence of overdispersion.

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

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

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)?

Only one ARIC paper has considered size subtypes of lacunes:
MS #1503. Bezerra et al. Risk factors for subtypes of cerebral infarct-like lesions detected by magnetic resonance imaging in the ARIC Study. Submitted

Other related publications:

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 primarily the result of an ancillary study (list number* ________)

Yes No
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.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:


