1.a. Full Title:

Specific Inflammatory Mediators Associated with Incident Ischemic Stroke and Individual Ischemic Stroke Subtypes

b. Abbreviated Title (Length 26 characters):

2. Writing Group:

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3. Timeline: 10/31/2016
4. **Rationale:**

Periodontitis is an infectious disease, which results in destruction of the tissues around the tooth surface and gingiva, loss of connective tissue attachment, erosion of alveolar bone, and tooth loss. The symptoms are bleeding gum (gingivitis), gingival pockets and bone loss (periodontitis) leading to tooth mobility and eventually tooth loss. Periodontitis is common and increases with age. In a US survey, about half of adults have some periodontitis and almost 10% have severe disease. Periodontitis is related to bacterial infection related systemic inflammation, which is implicated in the etiology of atherosclerosis leading to stroke, heart attack and other cardiovascular disease. Epidemiological studies suggest a link between periodontal disease and stroke. The linkage is derived from cross-sectional, case-control and cohort studies. More recent analyses from large-cohort studies suggest that there is a graded association between tooth loss and stroke, cardiovascular death, and all-cause mortality in patients with stable coronary artery disease. If causal, these associations would be of great importance because of the potential that preventing or treating periodontal disease could reduce the risk of major adverse cardiovascular events including stroke.

Periodontal disease (PD) is a risk factor for ischemic stroke. We propose to assess the hypothesis that specific inflammatory mediator(s) is associated with specific ischemic stroke subtypes in the Atherosclerosis Risk in Communities (ARIC) study. In 2009, Nambi et al studied the lipoprotein-associated phospholipase A2 and High-Sensitivity C-Reactive Protein (hs-CRP) in ischemic stroke risk in the Atherosclerosis Risk in Communities (ARIC) study in a cohort of less than 1000 subjects. The size of this cohort is a potential limitation. We propose a larger cohort study of 6000 subjects where hs-CRP and a more comprehensive panel of inflammatory markers was measured. Furthermore, we also propose to investigate the association between inflammatory markers and ischemic stroke subtype, not only ischemic stroke risk.

5. **Main Hypothesis/Study Questions:**

Is periodontal disease associated with increased risk of ischemic stroke? If so, are specific inflammatory mediator(s) associated with specific ischemic stroke subtypes?

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).

7. **Study design:**

In the ARIC study, serum inflammatory mediators were assessed in a cohort of subjects without prior stroke. They included high sensitivity C-reactive protein (hs-CRP), interleukin 1ra (IL-1ra), and Intercellular Adhesion Molecule 1(sICAM1) and were followed for all vascular events. All stroke events were adjudicated and classified into stroke subtypes by standard definitions. Multivariable Cox proportional hazards models will be used to study the relationship between elevated inflammatory markers (upper quartile compared with lower three quartiles) and ischemic stroke, as well as stroke subtypes (cardioembolic, lacunar or thrombotic).

**Limitation:**

1. Misclassification of ischemic stroke subtype: According to the algorithm, it requires the presence of a possible cardio-embolic source. Presence of a possible cardioembolic source may not necessarily mean cardioembolism as the etiology of the ischemic stroke. Also, artery-to-artery embolic stroke (e.g., dislodged carotid plaque) is classified in ARIC as "atherothrombotic". Lacunar stroke in ARIC is based on some imaging features, regardless of the presence or
absence of a "lacunar stroke syndrome". The definition may miss lacunar strokes with negative scans. Also, some lacunar strokes may be cardioembolic in etiology. Even though current classification does not allow for clear distinction between these subtypes within a stroke etiological type, we don’t necessarily expect misclassification to differ on the basis of migraine history.

2. Individual studies have limitations, which include the use of imprecise measures of periodontal disease, inadequate accounting for potential confounders, and low statistical power for vascular events relevant to the stroke/TIA population. Many of these issues were addressed in a prospective cohort study which evaluated the association between the presence of periodontal disease and recurrent vascular events in stroke/TIA patients. [18] One-hundred-six ischemic stroke or TIA patients were recruited at a single center. At enrollment, patients with high periodontal disease (defined as the highest tertile of attachment loss ≥ 5 mm and coinciding with initial and severe periodontitis) tended to be older, male, African-American, and to have lower education level and annual income; but, did not differ significantly by age, or prevalence of traditional risk factors including smoking, hypertension and cholesterol levels. Of these patients, 27 (26%) had recurrent composite vascular events over a median follow-up period of 24 months and 40 (38%) showed high periodontal disease. The associated Kaplan-Meier curve showed that a significantly higher proportion of patients with high periodontal disease experienced composite events of stroke/TIA/MI/death (47% compared to those who did not exhibit periodontal disease 19%; p=0.02). Periodontal disease was independently associated with composite vascular events (hazard ratio 2.8, 95% CI, 1.2-6.5), after adjustment for potential confounders, the association with high periodontal disease remained significant (hazard ratio 2.8, 95% CI, 1.0-8.0).

Despite the limitations, this will be the first study to evaluate association between periodontal disease and ischemic stroke risk, specifically, etiological stroke subtype risk. This proposal has important clinical implications and may help us better assess whether treatment of periodontal disease can reduce the rate of recurrent vascular events in patients with ischemic stroke or TIA.

Inclusion
Participants in the ARIC study who completed a fourth clinic examination (1996 to 1998) were assessed for periodontal disease. All participants at visit 4 will be included.

All stroke diagnoses (first and recurrent) are based on computer derived diagnosis and physician medical record review, with differences adjudicated by a second physician reviewer. Classification required evidence of sudden or rapid onset of neurological symptoms lasting >24 hours or leading to death, in the absence of evidence of a non-stroke cause. Strokes are further classified according to etiologic subtype as thrombotic brain infarction, lacunar infarction, cardioembolic stroke, ICH, or SAH according to criteria adopted from Nation Stroke Association.

Exclusion
Participants with missing periodontal information and/or serum inflammatory measures and those who do not meet the criteria as above will be excluded.

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 ICTDER 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

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)?

and high-sensitivity C-reactive protein improve the stratification of ischemic stroke risk
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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)* __________ __________)

*ancillary studies are listed by number at [http://www.cscc.unc.edu/aric/forms/](http://www.cscc.unc.edu/aric/forms/)

12a. 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.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is your responsibility to upload manuscripts to PUBMED Central whenever the journal does not and be in compliance with this policy. Four files about the public access policy from [http://publicaccess.nih.gov/](http://publicaccess.nih.gov/) are posted in [http://www.cscc.unc.edu/aric/index.php](http://www.cscc.unc.edu/aric/index.php), under Publications, Policies & Forms. [http://publicaccess.nih.gov/submit_process_journals.htm](http://publicaccess.nih.gov/submit_process_journals.htm) shows you which journals automatically upload articles to Pubmed central.