ARIC Manuscript Proposal Form

ARIC Publication Admin Use Only

Publication Committee Review Date: **06/10/25**
ARIC Manuscript Proposal Number: **# 4640**

1.a. Full Title: Oral Health Behavior, Oral infections, Systemic Inflammation, and Cardiovascular (CV) Outcomes

b. Abbreviated Title (Length 26 characters): Oral Health Behavior, infections, Inflammation, and CV Outcomes

2. Writing Group [please provide a middle initial if available; EX: Adam L Williams]:
Writing group members: Julie Marchesan, Souvik Sen, Kevin Moss, Jim Beck, Michelle Johansen, Kamakshi Lakshminarayan, Wayne Rosamond, Gustavo Giacomelli Nascimento

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

First author [please provide a middle initial; EX: Adam L Williams]:

Julie	**T**	**Marchesan**
First name	Middle initial	Last name

Address: UNC Adams School of Dentistry

Phone: 734-272-8962
E-mail: julie_marchesan@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 (The ARIC author should be involved enough in ARIC to be able to point the lead author to appropriate ancillary study PIs and to be able to search ARIC manuscript proposals if the lead author doesn't have the access needed to do such a search).

Name :Kimon Divaris and Wayne Rosamond
Address: UNC Adams School of Dentistry and Gillings School of Public Health

Phone:
E-mail: kimon_divaris@unc.edu and wayne_rosamond@unc.edu

3. Timeline: 6/1/25 – 5/30/26

4. Rationale: For many years the Dental Team from the ARIC dental ancillary study have proposed that oral infections are associated with all-cause mortality and CVD events. Recent studies have tied oral health behavior to reduction in rates of CV outcomes. [KOHH and NHANES] There have been some conflicting reports in the literature on these associations. One of the major issues in the dental research field is that there is currently no generally accepted definition of periodontal disease and other measures of oral health used for research. This is not unique to the periodontal field different definitions of the exposure can be found in many fields of medicine, especially in complex diseases. Periodontal literature uses both these subjective and objective definitions of disease. Our goal is to include objective and subjective definitions of oral health and identify the correlation of these definitions with CVD. All-cause mortality will be assessed, in addition to death by fatal coronary heart disease/myocardial infection, fatal coronary heart disease, myocardial infection, congestive heart failure, and ischemic stroke. Oral health-related definitions that we plan to investigate for correlation analysis and prediction of CVD include periodontal disease, caries, tooth loss/number, tooth brushing (presence/frequency) and oral health habits, such as flossing and dental visits (presence/frequency).

5. Main Hypothesis/Study Aims: 1) Oral health conditions are associated with all-cause mortality, incident CVD events and recurrent CVD events. 2) Oral health behaviors are associated with lowering of the rates of all-cause mortality, incident CVD events and recurrent CVD events 3) The effect of oral health behaviors on all-cause mortality, incident CVD events and recurrent CVD events is mediated by its' effects on oral health conditions including periodontal disease and caries induced inflammation measured as part of the dental ARIC study

6. Design and analysis:

a) **Inclusion/exclusion:** We will include ARIC participants who participated in the dental sub-study at Visit 4. Additional exclusion criteria will be 1) for incident CVD events we will exclude anyone with prevalent CVD at visit 4, 2) for recurrent CVD events we will only include those with prevalent CVD at visit 4.

b) **Study design:** We will use definitions of periodontal disease in accordance with disease classification by the American Academy of Periodontology, clinical measurements, and the Periodontal Profile Class as previously described by our group, caries, brushing frequency and flossing frequency, dental care utilization' data as our exposure and all-cause mortality and CVD events as our outcome(s). We will control for known risk factors of our outcome(s). Additionally, we will test if oral infection, conditions (periodontal disease and caries), inflammation (inflammatory markers measured part of dental ARIC) and CV risk factors (example atrial fibrillation), as possible mediators of effect of oral health behavior on CV outcomes. A directed acyclic graph (DAG) will be used to illustrate the proposed relationship.

c) Exposure, outcome and other variables of interest with specific reference to the time of their collection:

Oral health behavior is the exposure of interest. Dental flossing was assessed through the structured questionnaire administered in visit 4, that asked, "how often did you use dental floss last week" and the response was graded as "not at all", "one-time", 'two times" or 'three times or more". Those who reported as "not at all" were classified as non-flossers and all others as dental flossers. Tooth brushing was assessed through the structured question in visit 4 "How often did you brush your teeth yesterday?". Responses included "not at all", "one time", "two times", and "three times or more". Subjects were reported as regular brushers if they reported brushing their teeth ≥1 time a day. Dental care utilization was assessed through the question "Would you say that you use a dentist on a regular basis, or do you only go when you are in discomfort or when you need something fixed?". "Regular dental utilization" will be defined by the number of individuals that responded a "regular basis" and the remaining responses will define the category of Episodic dental utilization and included the following responses "only when in discomfort", "when something needs to be fixed", "don't go to the dentist", and "other".

We will treat ARIC Visit 4 as our baseline visit (the dental ancillary study was performed at visit 4). Age, sex, race (categorized as white and black, as well as race-center), smoking status, alcohol use, highest level of education, coronary artery disease (CAD), congestive heart failure (CHF) adjudication overseen the surveillance committee. Since smoking status is a strong confounder, we also plan to use pack-year of smoking as an additional covariate in the model. Body mass index was calculated as weight in kilograms divided by height in meters squared. Hypertension was defined as a systolic blood pressure of ≥140mmHg, a diastolic blood pressure ≥90mmHg, or use of medications to treat hypertension. Blood samples were obtained after individuals had fasted for 8 hours. Diabetes was determined by self-report of a physician diagnosis of diabetes, non-fasting blood glucose level of ≥200 mg/dL, fasting blood glucose level of ≥126mg/dL, or use of insulin or other oral hypoglycemic medications. Physical activity is considered significant if performed for 4hrs/week for at least a month. Prevalent CAD was defined by electrocardiographic evidence of previous myocardial infarction (MI), history of physician diagnosed MI, or previous coronary revascularization procedure (bypass, angioplasty). We will use the latest surveillance dataset for the longitudinal CV outcome(s)

d) Data analysis:

For the descriptive analysis, patient's demographic, medical history variables, and baseline clinical characteristics will be compared across oral infections (periodontal disease) and oral health behavior (presence/frequency of the habit of brushing, presence/frequency of the habit of flossing and dental care utilization) categories. Chi square testing and independent sample t-test will be used for comparison of categorical and continuous variables, respectively.

For survival evaluation (incidence and recurrence of events), Kaplan-Meier analyses with the log-rank test will be applied. Cox regression models will be used to calculate hazards ratios (HRs) of the effects of variables on the relationship between exposures and outcomes. We will also evaluate data with adjusted survival curves. Separate cox regression models will be used to calculate HRs and 95% CIs for association of

exposures with outcomes after adjustment for multiple covariates. Several models may be run including covariates --demographics (i.e. age, race, sex) and vascular risk factors (i.e. hypertension, cholesterol, diabetes, smoking). All statistical analyses will be two-tailed, and a P value of 0.05 will be considered statistically significant.

We propose to use sequential mediation analysis mediation pathway between oral health behavior and CV outcomes through oral health conditions and inflammation.
First, inflammatory markers (example CRP and IL6) will be standardized to have a mean of 0 and standard deviation of 1. Composite scores were then created for inflammation (mean of standardized CRP and IL6) and oral health (mean of PD using PPC stage and caries using DMFS). The mediation analysis tested three overall pathways:

1. Path A: Effect of oral health behavior on oral health condition using linear regression
2. Path B: Effect of oral health conditions on inflammation using linear regression
3. Path C: Effect of inflammation on CV outcome using logistic regression

To test the significance of the mediated effect, we will use Sobel test, which examines whether the indirect effect of oral health behavior on CV risk through the mediators (oral health conditions and inflammation) is significantly different from zero. The Sobel test statistic was calculated as the product of the coefficients divided by the standard error of the indirect effect. Other mediation methods will be applied, including IPTW (Inverse Probability of Treatment Weighting) and the g-formula as both methods can be used in causal inference to estimate the effect of an intervention on an outcome,

Missing data will be handled using complete case analysis, with potentially 5,000+ participants included in the final analysis after excluding participants with missing values on any analysis variable. All statistical tests were two-sided with significance set at $p < 0.05$. Results are presented as odds ratios with corresponding p-values to show both the magnitude and statistical significance of direct and indirect effects.

Exposure Variable

- Periodontal disease (PPC or periodontal profile class) assessed at V4
- Caries (DMFS-Decayed Missing Filled Surface) assessed at V4

Outcome Variables

- Composite CV outcome
- Composite ischemic stroke outcome
- All-cause mortality (including CV cause)
- Tooth loss as oral infection outcome

Covariates

Age
Sex
Race
Field Center
Hypertension
Atrial Fibrillation
Diabetes
Obesity
Smoking
Alcohol intake
Physical activity (if available for visit 4)

Diet (if available for visit 4)

Mediators

- Inflammatory markers (CRP, IL-6, others measured as part of DARIC V4)
- Oral infection status for specific mediation pathway (Behavior→Outcome)

e) Any anticipated methodologic limitations or challenges if present

1. There is a possibility that oral health behavior is associated with other health behavior such as physical activity and good diet leading to selection confounding bias. In that case we propose to use propensity score matching (PSM) to generate balanced groups to test outcomes **2**. There is a possibility that there are unmeasured confounders that may not be included in the analysis. E-values will be used to assess unmeasured confounding. We propose to run sensitivity analysis to test the effect of such unmeasured confounders

f) Will the author need Limited data to complete the proposed manuscript? ☐ Yes, Limited data is needed (Provide a brief (2-3 sentences) justification for requesting PHI data) . ☒ No, De-identified data will be sufficient.

*Please note, Limited dataset access is strict and rarely provided. Limited data includes identifiable information such as dates (birthdays, visit dates, etc.). CMS, Genomic, Geocoded, Proteomics/Somalogic, and other -omic data all fall under the limited data category. De-identified data does not include dates. All dates are date adjusted to "Days since Visit 1".

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? (Non-ARIC analysis means that the authors are not regarded as ARIC investigators and the "ARIC author" is essentially just a facilitator rather than an integral part of the writing group.) ☐ Yes ☒ No

b. If Yes, is the author aware that the current derived consent file ICTDER05 must be used to exclude persons with a value RES_OTH and/or RES_DNA = "ARIC only" and/or "Not for Profit" ? ☒ Yes ☐ No
(The file ICTDER is distributed to ARIC PIs annually, 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 current derived consent file ICTDER05 must be used to exclude those with value RES_DNA = "No use/storage DNA"? ☒ Yes ☐ No

9. The lead author or the "sponsoring" ARIC 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 website at:

https://aric.cscc.unc.edu/aric9/proposalsearch [ARIC Website□Publications□Proposal Search]

☒ 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)?

There are many manuscript proposals that use dental variables as an exposure including but not limited to #492, 687, 861, 730, 827, 858, 913, 915, 929, 995, 1112, 1284, 1892, 2053 and 1859.

The following used specific CV outcomes and stroke: 2886, 3080, 3472 and 3790

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or does it use current [or ongoing] ancillary study data (this includes ACHIEVE)? ☒ Yes ☐ No → Skip to question 12

11.b. If yes to 11.a., is the proposal

☒ **A. primarily the result of an ancillary study**

☐ **B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables)**

11.c. If yes to 11.a., list number* 1996.01__

*ancillary studies are listed by number
https://aric.cscc.unc.edu/aric9/researchers/ancillary_studies/approved_ancillary_studies [ARIC Website□Ancillary Studies□Approved Ancillary Studies]

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/ are posted in https://aric.cscc.unc.edu/aric9/publications/policies_forms_and_guidelines [ARIC Website□Publications□Publication Policies, Forms, and Guidelines]. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

1. References: Yu Y-H, Steffensen B, Chasman DI, Buring JE. Self-reported oral health is associated with systemic health outcomes and all-cause mortality J Am Dent Assoc. 2024;155(3):233-243.e8. doi: 10.1016/j.adaj.2023.11.006.

2. Janket SJ, Lee C, Surakka M, Jangam TG, Van Dyke TE, Baird AE, Meurman JH. Oral hygiene, mouthwash usage and cardiovascular mortality during 18.8 years of follow-up Br Dent J. 2023:1-6. doi: 10.1038/s41415-023-5507-4.

3. Sen S, Logue L, Logue M, Otersen EAL, Mason E, Moss K, Curtis J, Hicklin D, Nichols C, Rosamond WD, Gottesman RF, Beck J. Dental Caries, Race and Incident Ischemic Stroke, Coronary Heart Disease, and Death. Stroke. 2024;55(1):40-49. doi: 10.1161/STROKEAHA.123.042528. Epub 2023 Nov 29. PMID: 38018831; PMCID: PMC10841981.

4. Wang H, Divaris K, Pan B, Li X, Lim JH, Saha G, Barovic M, Giannakou D, Korostoff JM, Bing Y, Sen S, Moss K, Wu D, Beck JD, Ballantyne CM, Natarajan P, North KE, Netea MG, Chavakis T, Hajishengallis G. Clonal hematopoiesis driven by mutated DNMT3A promotes inflammatory bone loss. Cell. 2024 Jul 11;187(14):3690-3711.e19. doi: 10.1016/j.cell.2024.05.003. Epub 2024. PMID: 38838669

5. Sen S, Meyer J, Mascari R, Trivedi T, Suri F, Wasserman B, Rosamond W, Moss K, Beck J, Gottesman RF. Association of Dental Infections with Intracranial Atherosclerotic Stenosis. Cerebrovasc Dis. 2024;53(1):28-37. doi: 10.1159/000530829. Epub 2023. PMID: 37121226.

6. Adam HS, Lakshminarayan K, Wang W, Norby FL, Mosley T, Walker KA, Gottesman RF, Meyer K, Hughes TM, Pankow JS, Wong DF, Jack CR Jr, Sen S, Lutsey PL, Beck J, Demmer RT. The prospective association between periodontal disease and brain imaging outcomes: The Atherosclerosis Risk in Communities study. J Clin Periodontol. 2022 Apr;49(4):322-334. doi: 10.1111/jcpe.13586. Epub 2022 Jan 27. PMID: 34905804; PMCID: PMC8934294.

7. Marchesan JT, Morelli T, Moss K, Preisser JS, Zandona AF, Offenbacher S, Beck J. Interdental Cleaning Is Associated with Decreased Oral Disease Prevalence. J Dent Res. 2018 Jul;97(7):773-778. doi: 10.1177/0022034518759915. Epub 2018 Feb 26. PMID: 29481764; PMCID: PMC6728587.

8. Marchesan JT, Byrd KM, Moss K, Preisser JS, Morelli T, Zandona AF, Jiao Y, Beck J. Flossing Is Associated with Improved Oral Health in Older Adults. J Dent Res. 2020 Aug;99(9):1047-1053. doi: 10.1177/0022034520916151. Epub 2020 Apr 22. PMID: 32321349; PMCID: PMC7375740.

To view publications materials, click "Log in" at the top right of the ARIC website. Click "Forgot Password" if you are experiencing issues with logging in.