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Periodontal and other oral bacteria and risk of lung cancer in the Atherosclerosis Risk in Communities (ARIC) Study

Baijun Zhou[1], **Jiayun Lu**[1], **James D. Beck**[2], **Kevin L. Moss**[2], **Anna E. Prizment**[3], **Ryan T. Demmer**[4], **Kori A. Porosnicu Rodriguez**[1,5], **Corinne E. Joshu**[1,6], **Dominique S. Michaud**[7], **Elizabeth A. Platz**[1,6]

[1]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

[2]Division of Comprehensive Oral Health/ Periodontology, Adams School of Dentistry, University of North Carolina, Chapel Hill, NC

[3]Division of Hematology, Oncology and Transplantation, University of Minnesota Medical School, and the University of Minnesota Masonic Cancer Center, Minneapolis, MN

[4]Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN

[5]Johns Hopkins University School of Medicine, Baltimore, MD

[6]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD

[7]Department of Public Health and Community Medicine, Tufts University School of Medicine, Boston, MA

Abstract

Background: Evidence suggests that periodontal disease is associated with increased lung cancer risk, but whether periodontal pathogens are explanatory is unknown. We prospectively studied associations of pre-diagnostic circulating antibodies to oral bacteria and of periodontal bacteria in subgingival plaque with lung cancer.

Methods: We included 4,263 cancer-free participants in the Atherosclerosis Risk in Communities study with previously measured serum IgG antibodies to 18 oral bacteria. In 1,287 participants for whom subgingival plaque was collected, counts for 8 periodontal bacteria were previously measured. Incident lung cancers (N=118) were ascertained through 2015 (median follow-up=17.5 years). We used Cox regression to estimate multivariable-adjusted associations, including for sums of antibodies to orange (*C. rectus*, *F. nucleatum*, *P. intermedia*, *P. micra*, *P. nigrescens)* and red (*P. gingivalis*, *T. forsythensis*, *T. denticola*) complex bacteria.

Results: Orange complex bacteria antibodies were positively associated with lung cancer (per IQR HR=1.15, 95% CI 1.02–1.29), which was stronger in men (HR=1.27, 95% CI 1.08–1.49), and explained by *P. intermedia* and *P. nigrescens* (HR=1.15, 95% CI 1.04–1.26). Suggestive positive

Corresponding author: Elizabeth A. Platz, ScD, MPH, Department of Epidemiology, Rm E6143, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St. Baltimore, MD 21205; tel: 410-614-9674; eplatz1@jhu.edu.

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associations with lung cancer (N=40) were observed for *F. nucleatum*, *A. actinomycetemcomitans*, and *P. gingivalis* counts. Significant positive associations were found for count to antibody ratio for *P. intermedia* and *P. gingivalis*.

Conclusions: We identified positive associations with lung cancer for oral bacteria, especially orange complex which are moderately pathogenic for periodontal disease.

Impact: This prospective study supports the need for more research on periodontal bacteria in lung cancer etiology. If associations are supported, this may inform novel lung cancer prevention strategies.

Keywords

lung cancer; bacteria; periodontal disease; risk; cohort

Introduction

Evidence suggests that periodontal disease, which is highly prevalent in the US (1), is associated with lung cancer risk (2, 3), even accounting for smoking (3–5), a cause of both periodontal disease and lung cancer. Periodontal disease can result from infection and inflammation of the tissues that surround and hold the teeth in place (6). Whether periodontal pathogens linked with periodontitis or other oral bacteria may explain the link between periodontal disease and lung cancer risk is unknown. Oral bacteria could influence lung cancer risk via lung aspiration causing infection and an inflammatory response, together initiating and/or promoting carcinogenesis (7). Another potential mechanism is that the systemic immune response to oral microbiota affects cancer risk and tumor surveillance (7).

Socransky et al. categorized oral bacteria into 5 complexes based on their statistical co-occurrence in subgingival plaque (8). The role of these complexes in dental plaque formation and their periodontal disease pathogenicity differ (8). For example, orange complex bacteria (e.g., *P. intermedia*, *P. nigrescens*, *F. nucleatum*) are strongly related to increased depth of the pocket between a tooth and the gum, a clinical measure of periodontal disease. Red complex (e.g., *P. gingivalis*, *T. forsythensis*, *T. denticola*) are considered to be highly pathogenic, in that they drive damage to the tissue and bone that anchor the teeth. Whether these subgingival bacterial complexes that differ in pathogenicity for periodontal disease are also differentially related to lung cancer remains to be determined.

Few prospective cohort studies have investigated the association between periodontal bacteria or circulating antibodies to those bacteria and lung cancer risk. A cohort study reported a borderline association between presence of any of three orange complex-associated oral pathogens detected as bacterial DNA in dental plaque (*F. nucleatum*, *P. intermedia*, *C. rectus*) and lung cancer incidence (9). In contrast, Shi et al. reported that the abundance of the orange complex bacterium *P. micra* was lower in lung cancer cases than controls nested in a prospective cohort study (10). Further, they reported a possible positive association between presence of *A. actinomycetemcomitans* and lung cancer risk, but no positive associations for presence of four other pre-specified periodontal pathogens,

P. gingivalis, T. forsythia, and *T. denticola* (red complex), or *P. intermedia* (orange complex), in mouth rinse and lung cancer risk (10). For other cancers, higher circulating level of antibodies to *P. gingivalis* (red complex) was associated with increased orodigestive cancer mortality among participants without apparent periodontal disease (11) and with increased pancreatic cancer risk (12).

Given emerging evidence for periodontal disease and limited studies for oral bacteria, we further investigated the role of oral bacteria in lung cancer etiology by prospectively examining the associations of serum IgG antibody levels for 18 periodontal and other oral bacteria, and counts for 8 periodontal bacteria, individually and summed in Socransky's complexes (8), and lung cancer risk. We conducted this work in the Atherosclerosis Risk in Communities (ARIC) study, a cohort of mostly Black and White men and women, in which we observed that severe periodontal disease was associated with increased lung cancer risk after accounting for smoking (3).

Materials and Methods

Study Population

The ARIC study (RRID:SCR_021769) is a prospective cohort of 15,792 mostly White and Black men and women aged 45–64 years enrolled between 1987–1989 from four communities (Forsyth County, NC; Jackson, MS, Minneapolis, MN; and Washington County, MD) (13). Participants received a physical examination and were re-examined every three years (13) through visit 4 in 1996–1998, and then were examined at four more visits starting in 2011–2013. A clinical dental examination was performed at visit 4. Of 11,656 participants who attended visit 4, 60% were eligible (i.e., had at least 1 natural tooth or implant, did not have dental probing as a contraindication) and consented to dental examination. Among the dental examination participants (N=6,793), we excluded those with a cancer history before visit 4, Black participants from the Minneapolis and Washington County field centers (numbers too small to adjust for race by field center), and participants who are not Black or White. We included 4,263 participants in the oral bacteria antibody analytical cohort after further excluding those without antibody information for all 18 bacteria. Of the subset of 1,450 participants for whom subgingival plaque was collected, we included 1,287 participants in the analytical cohort for bacteria count after further excluding those without count information for all 8 bacteria.

The ARIC participants gave written informed consent. The Institutional Review Boards at each study site approved the ARIC study protocol and the research was conducted under the U.S. Common Rule.

Measurement of antibodies to oral bacteria

Serum concentrations of immunoglobulin (IgG) antibodies to 18 oral bacteria were previously measured in ARIC (14) by checkerboard immunoblotting (15): *Aggregatibacter actinomycetemcomitans*, *Actinomyces viscosus*, *Capnocytophaga ochracea*, *Campylobacter rectus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Helicobacter pylori*, *Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens*, *Parvimonas micra*, *Streptococcus*

intermedius, *Seleomonas noxia*, *Streptococcus oralis*, *Streptococcus sanguis*, *Tannerella forsythensis*, *Treponema denticola*, and *Veillonella parvula*. These bacteria are known or suspected to be associated with periodontal disease (8), except *Helicobacter pylori*, the bacterium that causes stomach cancer and for which the oral cavity is a reservoir. We included *H. pylori* because a meta-analysis suggested that seropositivity is associated with lung cancer (16), although the quality of the evidence is considered to be very low at the time of the current analysis (17). The IgG limit of detection (LOD) was 20 ng/mL; values were reported for participants with concentrations below LOD, and these reported values were used in the statistical analyses of continuous antibodies and sums of antibodies.

Measurement of DNA-derived bacteria counts

Subgingival plaque that was collected from the mesial site of the maxillary right first molar for a random subset of participants during the dental examination (21.3%) was used. Participants who required antibiotics before a dental examination were not eligible for this collection. Microbial DNA was previously measured in the dental plaque (18) using a modification (19) of the checkerboard DNA-DNA hybridization method from Socransky et al. (20) for 8 oral bacteria selected because they are periodontal pathogens: *Aggregatibacter actinomycetemcomitans*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens*, *Tannerella forsythensis*, and *Treponema* denticola (18). Using this method, bacteria counts were derived using established standards, and the count LOD was 10^4 (18); values were reported for participants with counts <LOD, and these reported values were used in the statistical analyses. Bacteria counts were not standardized to the amount of plaque collected from each participant (the more plaque present the greater the bacterial mass) (18).

Ascertainment of incident lung cancer cases

The primary analysis outcome was a first primary lung cancer diagnosis after visit 4 through 2015. Other outcomes were first primary non-small cell lung cancer (NSCLC), and lung cancer mortality. Lung cancer cases were ascertained by linkage to the cancer registries in the four states where the participants were recruited and supplemented with medical records, hospital discharge summaries, and death certificates (21). Lung cancer deaths were identified from death certificate (underlying cause) and linkage with the National Death Index (21).

Measurement of covariates

Covariates were collected during clinical examinations and on annual follow-up calls. We included risk factors for periodontal disease and/or lung cancer as covariates assessed at visit 4 (unless otherwise indicated): age, field center, race (Black or White), ever use of hormone replacement therapy (HRT; females), body mass index (BMI), cigarette smoking status (never, former, current) and packyears, alcohol drinking status (never, former, current), diabetes status, and family history of cancer (visit 2,3). BMI was calculated from weight and height measured by trained staff. Packyears were calculated from duration and number of cigarettes. Participants who reported a physician diagnosis of diabetes or reported diabetes treatment at any visit were defined as having diagnosed diabetes. We classified participants without diagnosed diabetes as having undiagnosed diabetes (fasting glucose ≥126 mg/dL,

non-fasting glucose ≥200 mg/dL, or glycated hemoglobin ≥6.5% (visit 2)), as being at risk of diabetes (fasting glucose 100 to <126 mg/dL or non-fasting glucose of 140 to <200 mg/dL), or as not having diabetes/not at risk for diabetes. We also included calculated lifecourse socioeconomic status (SES) (22) and SES-related covariates collected at visit 4: having a dentist, frequency of dental visits, time since last dental visit, health insurance status, medical visits frequency.

Statistical analysis

Analyses were performed using SAS 9.4 (Cary, NC). Statistical tests were 2-sided. $P<0.05$ was considered to be statistically significant. We did not perform correction for multiple testing for this candidate approach.

Antibodies to oral bacteria—We summarized baseline participant characteristics by antibody concentration >LOD or ≤LOD to *P. gingivalis*, a prime etiologic agent in periodontal disease (23). Summary statistics for BMI, cigarette smoking status, packyears, and drinking status were adjusted for age and race. We tested for differences in these characteristics using the t-test (continuous variables), chi-square test (categorical variables), or Wald test (adjusted variables). We compared the median concentration of pre-diagnostic antibodies in lung cancer cases and non-cases using the Wilcoxon rank-sum test.

Multivariable-adjusted Cox proportional hazards regression was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for associations between concentration of antibodies to each oral bacterium and lung cancer risk. Participants contributed person-time at risk until diagnosed with lung cancer, diagnosed with another cancer, died, or end of follow-up for this analysis (12/31/2015). Antibody concentrations >LOD were entered in the model as two indicator variables with the cut-point at the median (middle, high groups); the reference (low group) was an antibody concentration ≤LOD. To test for trend, we entered a single ordinal variable with the median antibody concentration for the low, middle, and high groups; the coefficient was tested using the Wald test. In model 1, we adjusted for age, joint terms for field center and race (Black from Jackson; Black from Forsyth; White from Forsyth; White from Washington County [reference: White from Minneapolis]), and joint terms for sex and HRT use (female user, female nonuser [reference: male]). In model 2, we additionally adjusted for cigarette smoking, packyears, alcohol drinking, BMI, diagnosed diabetes, undiagnosed diabetes, at risk for diabetes, family history of cancer, and used inverse probability weighting by a propensity score to control for lifecourse SES and SES-related factors. We predicted the propensity score by modeling the association between antibody concentration and lifecourse SES, having a dentist, frequency of dental visits, time since last dental visit, health insurance status, and medical visit frequency using logistic regression. We imputed missing values for packyears (N=353) and family history of cancer (N=184) using the fully conditional specification method of multiple imputation (24) (Supplement Method 1). Similar analyses were performed to investigate NSCLC (with censoring at date of diagnosis of a different histology lung cancer) and lung cancer death (participants contributed person-time at risk until death from lung cancer, death from another cause, or end of follow-up). The proportional hazards assumption was tested by including

two interaction terms for the middle and high antibody level groups with follow-up time and testing their coefficients using the Wald test.

To investigate whether bacterial complexes that tend to co-occur in subgingival plaque are associated with lung cancer, we summed antibody concentrations for red (*P. gingivalis*, *T. denticola*, *T. forsythensis*) and orange/orange-related (*C. rectus*, *F. nucleatum*, *P. intermedia*, *P. micra*, *P. nigrescens*) complex bacteria (8). To investigate whether a history of exposure to any versus specific oral bacteria are related to lung cancer, we summed antibody concentrations for all 18 bacteria. We entered these sums as continuous variables into the model. We assessed linearity of the continuous associations using restricted cubic splines and tested for linearity using the likelihood ratio test.

In sub-analyses, we restricted to non-diabetics, ever smokers (few lung cancer cases in never smokers) and stratified by follow-up time (at the median among the cases), sex, and race. We tested for effect modification by sex or race using likelihood ratio tests.

DNA-derived counts of periodontal bacteria—We estimated the association between DNA-derived bacteria counts (individual bacteria, red and orange complex sums, total sum) and lung cancer risk using Cox proportional hazards regression adjusting for the same covariates as for the antibody analyses. Counts >0 were entered in the model as two indicator variables with the cut-point at the median count (middle, high groups); the reference was not detected. We also modeled counts as continuous and binary (detected vs not detected) variables. We also modeled the ratio of DNA-derived bacteria counts to antibody concentration (both after log transformation) for the 8 periodontal pathogens, and their joint associations (4 categories: ≤LOD/not detected, ≤LOD/detected, >LOD/not detected, >LOD/detected).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request (https://sites.cscc.unc.edu/aric/pubs-policies-and-forms-pg).

Results

Antibodies to oral bacteria and lung cancer risk

We ascertained 118 first primary lung cancer cases in 63,321 person-years over a median of 17.5 years of follow-up. Mean age in the cohort was 62 years, 16.3% were Black, and 55.0% were female. Participants with *P. gingivalis* antibodies >LOD were more likely to be Black, to be less educated, to have a diabetes diagnosis, and to be obese (Table 1). The extent of correlation among the antibodies to the 18 oral bacteria was variable; for example, *P. intermedia* and *P. nigrescens* (both orange complex) were highly correlated (Spearman r=0.8), whereas *P. gingivalis* (red complex) and *P. micra* (orange complex) were weakly correlated (Spearman r=0.2).

Pre-diagnostic antibody concentration distributions (unadjusted) among the lung cancer cases and non-cases were generally similar with a few exceptions (Supplement Figure 1). The proportion of the cohort with antibody concentrations ≤LOD ranged from 20.0% for

A. actinomycetemcomitans to 83.1% for *V. parvula*; proportions were generally similar between lung cancer cases and non-cases (Supplement Table 1). Median pre-diagnostic antibody concentrations did not statistically significantly differ between lung cancer cases and non-cases, except for *P. micra*, for which the median antibody concentration was higher among cases (P=0.01; Table 2).

No statistically significant associations with lung cancer risk were observed for antibodies in the middle or higher groups compared with the ≤LOD group for the 18 oral bacteria (Table 3). However, when modeled as a continuous variable, the antibody sum for the 5 orange complex bacteria was statistically significantly positively associated with lung cancer risk (per IQR: HR=1.15, 95% CI 1.02–1.29; Table 4). This association was explained by antibodies to *P. intermedia* and *P. nigrescens*: their sum was positively associated with lung cancer risk (per IQR: HR=1.15, 95% CI 1.04–1.26; Table 4); the HR was consistent when modeling the sum of decile ranks for these antibodies (per IQR: HR=1.14, 95% CI 0.81–1.60). In contrast, the HR for the antibody sum for the other 3 orange complex bacteria was attenuated and not significant. Associations for the sum of the orange complex bacteria antibodies (P=0.54) and sum of the *P. intermedia* and *P. nigrescens* antibodies (P=0.42) did not differ from linearity (Supplement Figure 2). Associations of total, orange complex bacteria, *P. intermedia* and *P. nigrescens* with lung cancer were notably stronger in the stratum with shorter (≤9.9 years) than longer follow-up time (Table 4). The association for the red complex bacteria was null in both the shorter and longer follow-up time strata.

We found stronger positive associations for the sum of the orange complex bacteria antibodies (per IQR: HR=1.27, 95% CI 1.08–1.49) and sum of the *P. intermedia* and *P. nigrescens* antibodies (HR=1.22, 95% CI 1.07–1.38) with lung cancer risk among men than overall, whereas the associations among women were null or weak, respectively (Table 4); this sex difference was not significant ($P_{\text{interaction}}$=0.13), however. A positive association for the sum of *P. intermedia* and *P. nigrescens* antibodies with lung cancer was observed among Black (per IQR: HR=1.22 95% CI 1.00–1.48) and White (HR=1.13, 95% CI 1.01–1.26) participants (Table 4). When modeling associations using three antibody concentration groups, most associations among men and women were not statistically significant, including for the sum of the antibodies for the orange complex bacteria (tertiles) and for *P. intermedia* and *P. nigrescens* (≤LOD, and two groups >LOD). Nevertheless, these HRs among males tended to be positive and larger than overall (Supplement Table 2). Associations for the three antibody concentration groups among Black and White participants were not significant, with a few exceptions. However, the HRs among White participants tended to be the same as or stronger than overall, whereas in Black participants, associations were null or appeared inverse, including for the orange complex bacteria and *P. intermedia* and *P. nigrescens,* albeit based on few lung cancer cases (Supplement Table 2).

Among ever smokers (60.0% of the analytic cohort), the patterns were similar to overall, including for the sum of orange complex and sum of *P. intermedia* and *P. nigrescens* antibodies, but associations were not significant (Supplement Table 3). Among non-diabetic participants (50.8% of the analytic cohort), the sums of the antibodies to orange complex bacteria (per IQR: HR=1.24, 95% CI 1.05–1.45) and to *P. intermedia* and *P. nigrescens* (HR=1.28, 95% CI 1.14–1.44) were significantly positively associated with lung cancer risk

(Supplement Table 3). Other associations were similar to overall. Comparable patterns of association were observed for risk of NSCLC as for total lung cancer, including for the sum of antibodies to the orange complex bacteria (per IQR: HR=1.20, 95% CI 1.06–1.36; Supplement Table 4). For lung cancer mortality, associations were not significant and mostly similar to those for incidence (Supplement Table 4).

DNA-derived bacteria counts and lung cancer risk

In the subset of eligible participants in whom subgingival plaque was collected, 40 first primary lung cancer cases occurred in 18,808 person-years of follow-up. Of the DNA-derived counts for the 8 oral bacteria, the percentage for which the bacterium was not detected ranged from 24.0% (*A. actinomycetemcomitans*) to 34.1% (*P. intermedia*). Only 0.54% of participants had all 8 of the bacteria not detected in their plaque, while 26.4% had all of the bacteria detected. Median pre-diagnostic DNA-derived counts did not differ between the lung cancer cases and controls (Supplement Table 5). The associations of DNA-derived counts for the 8 oral bacteria, and sums of the orange complex, red complex, or total bacteria with lung cancer risk were not statistically significant, with the exception of an inverse association for *T. forsythensis* (red complex) that was statistically significant in the middle group (Table 5). However, suggestive positive associations were observed for *F. nucleatum* (detected vs not detected: HR=2.27, 95% CI 0.93–5.55), an orange complex bacterium, *A. actinomycetemcomitans* (detected vs not detected: HR=1.99, 95% CI 0.76–5.19), and *P. gingivalis* (vs not detected: middle category HR=1.94, 95% CI 0.81–4.61; top category HR=1.47, 95% CI 0.62–3.52), a red complex bacterium (Table 5).

Because antibodies reflect history of infection, but do not differentiate between current and past infection, and bacterial DNA reflects current colonization, and thus may be reflecting different exposure time points, to inform differences in their associations with lung cancer risk, we assessed their ratios and joint distributions for the 8 periodontal pathogens. Overall, the distributions of antibody concentrations did not differ between participants with and without detected bacterial DNA (Supplement Table 6). However, the ratios of DNA-derived bacteria count to antibody concentration (both after log transformation) for *P. intermedia* (per IQR: HR=1.93, 95% CI 1.10–3.36) and *P. gingivalis* (per IQR: HR=1.71, 95% CI 1.16–2.50) were positively associated with lung cancer risk (Supplement Table 7). The association was U-shaped for *T. forsythensis* (versus middle tertile: lowest tertile HR=3.31, 95% CI 1.26–8.68, highest tertile HR=2.64, 95% CI. 1.01–6.95) (Supplement Table 7). For joint categories of antibodies (>LOD vs ≤LOD) and DNA-derived bacteria counts (detected vs not detected), compared with being ≤LOD/not detected, the HR of lung cancer was non-statistically significantly elevated for all three combinations for *F. nucleatum*, especially for being >LOD/detected (HR=3.21, 95% CI 1.00–10.29; Supplement Table 8).

Discussion

In this prospective study, we identified positive associations with lung cancer risk for orange complex bacteria, which co-occur in subgingival plaque, are pro-inflammatory, and are moderately pathogenic for periodontal disease. These findings were based on both antibodies (*P. intermedia* and *P. nigrescens*) and bacteria counts (*F. nucleatum*). Suggestive

positive associations with lung cancer risk were also observed for bacteria counts for *A. actinomycetemcomitans* and *P. gingivalis.* Significant positive associations were also found for the ratio of bacteria count to antibodies for *P. intermedia* (orange complex) and *P. gingivalis,* a prime etiologic agent (red complex) in periodontal disease (23). These associations were independent of cigarette smoking, a strong risk factor for both periodontal disease and lung cancer. These findings support the need for more research on periodontal disease and periodontal pathogens in lung carcinogenesis.

To our knowledge, this is the first study to investigate the association between antibodies to 18 oral bacteria, including periodontal pathogens, and lung cancer risk. We focused on lung cancer because prospective cohort studies have reported positive associations between periodontal disease and lung cancer incidence or mortality (4, 5, 25), including in ARIC (3). We identified a positive association, which was compatible with a linear dose-response, between the sum of the antibodies to the 5 orange complex bacteria that were measured in ARIC – *C. rectus*, *F. nucleatum*, *P. intermedia*, *P. micra*, and *P. nigrescens* – and lung cancer risk. This association was explained by *P. intermedia* and *P. nigrescens.* Associations were stronger for antibodies to the orange complex bacteria for follow-up within 10 years of blood collection. We also observed stronger positive associations in men and in White participants than overall, which aligns with our prior findings in ARIC for periodontal disease and lung cancer (3). Associations for antibodies to orange complex bacteria were stronger for NSCLC than overall, and present but not significant for lung cancer mortality.

In ARIC participants who did not require antibiotic treatment before dental examination and who had subgingival plaque collected, DNA-derived counts for orange complex bacteria and for *P. intermedia* and *P. nigrescens* were not associated with lung cancer, although detected DNA- for the orange complex bacterium *F. nucleatum* was suggestively positively associated, with more than twice the risk (HR=2.27, 95% CI 0.93–5.55). The size of the analytic cohort (40 cases in 1,287 participants) precluded investigating associations by sex and race. Our findings appear to be consistent with those from the Mai et al. prospective cohort study of 1,200 women with 17 subsequent lung cancer cases in which a positive association between the presence of any of three orange-complex pathogens – *F. nucleatum*, *P. intermedia*, *C. rectus* – detected as bacterial DNA in dental plaque with lung cancer risk was found (HR=3.02, 95% 0.98–9.29) (9). Of these, the association (non-significant) was most apparent for *F. nucleatum* (HR=2.27, 0.73–7.03) (9). In a study of 156 lung cancer cases and 156 controls nested in the Southern Community Cohort Study, Shi et al. reported no association for the presence of *P. intermedia*, the only orange complex oral pathogen they pre-specified, with lung cancer risk and a lower abundance of *P. micra* (orange complex) in cases than controls; bacteria were detected by 16S rRNA gene sequencing in pre-diagnostic mouth rinse samples (10). Shi et al. did not report on *F. nucleatum* or *C. rectus.*

Taken together, the findings for circulating antibodies from ARIC and DNA-derived bacteria counts in subgingival plaque from the Mai et al. study (9) and ARIC suggest a role for orange complex bacteria in lung cancer etiology independent of smoking. Given the strong link between orange complex bacteria and periodontal disease (8), these findings may possibly, in part, explain the positive association between periodontal disease and lung cancer observed in ARIC. Orange complex bacterial species have been detected in

systemic infections and inflammation, including rheumatoid arthritis, inflammatory bowel disease and colorectal cancer, and lung abscesses (26, 27). Among the orange complex bacteria, *F. nucleatum* was most prevalent in extra-oral infection sites, including in colorectal adenocarcinoma (28). In our study, antibodies to orange complex species *P. intermedia* and *P. nigrescens* were positively associated with lung cancer, although not antibodies to *F. nucleatum*, *P. micra,* or *C. rectus*. With respect to biological plausibility, oral bacteria may reach the lung by aspiration or by entering the circulatory system from ulcerated periodontal pocket walls (29). Tissue infections by oral bacteria, including in the lung, can lead to chronic low-grade inflammation, which, then, can promote carcinogenesis (30). Inflammation, including infection-associated, is well recognized as a cause of stomach and colon cancers (31). Another possible mechanism is that oral bacteria alter the immune system (e.g., by eliciting autoimmunity) and potentiate cancer development (e.g., by eliciting systemic pro-inflammatory responses) (32).

Addressing the link between periodontal disease and cancer, prior studies have reported that antibodies to *P. gingivalis* were positively associated with other cancers, specifically orodigestive cancer mortality (11) and pancreatic cancer risk. In ARIC, antibodies to *P. gingivalis* were not associated with lung cancer, whereas DNA-derived count was suggestively positively associated, and notably the count to antibody ratio was statistically significantly positively associated. In prospective studies, Mai et al. (9) reported no association between the presence of *P. gingivalis* in dental plaque and Shi et al. (10) reported a possible inverse association for *P. gingivalis* in dental rinse with lung cancer risk. We also noted that participants detected DNA for *A. actinomycetemcomitans*, a bacterium linked with aggressive periodontitis, appeared to have twice the lung cancer risk, albeit not statistically significant. The HR of lung cancer for antibodies to this agent was 1.28 in the highest category and not statistically significant. Shi et al. also noted a possible, more than two-fold increased lung cancer risk for the presence of *A. actinomycetemcomitans* in mouth rinse samples (10). Counts for another red complex bacterium *T. forsythensis* appeared to be inversely associated, although this pattern was not seen for antibodies to this bacterium. Shi et al. reported no association for the presence of *T. forsythensis* in mouth rinse samples and lung cancer risk although the OR was also less than 1.0 (10). Associations with lung cancer were not observed for antibodies to the other bacteria, including *H. pylori*, or for counts for the other periodontal bacteria in ARIC. An additional prospective study investigated the association between the diversity of the oral microbiome in oral rinse samples and risk of lung cancer in never smokers in a nested case-control study in the Shanghai Women's Health Study and the Shanghai Men's Health Study (33). They identified associations with lung cancer risk for six taxa, some inverse and some positive. While our studies are not directly comparable given methodologic and population differences, both studies suggest the need for further work addressing the oral microbiome, including periodontal pathogens, and lung cancer risk.

Several aspects of the study warrant discussion. First, antibody concentrations were measured only once. While antibody titers remain relatively stable over 30 months (34), median follow-up was 17.5 years. Thus, we cannot rule out error due to temporal variability in oral bacterial infection or antibody production leading to null results for some bacteria. Support for this contention comes from the associations for the sum of the orange complex

bacteria being substantially stronger within the first 10 years of blood collection. Second, some of these bacteria may be present elsewhere in the body (e.g., gastrointestinal tract), thus, antibody concentrations may not reflect current or past colonization of the oral cavity. Third, we did not observe the same orange complex bacteria as being associated with lung cancer for antibodies (*P. intermedia, P. nigrescens*) and bacteria counts (*F. nucleatum*). However, these two measures do not necessarily reflect the same time point of subgingival infection: we used circulating antibodies as an indicator of infection history, and bacteria counts in subgingival plaque as an indicator of current colonization. Indeed, we observed that antibody concentrations did not differ between participants with and without detectable DNA for the same bacteria.

Fourth, higher antibody concentrations may not be interpretable as greater or longer subgingival infection. Circulating antibody concentrations reflect a complex combination of the fact and duration of infection, initial innate and subsequent (adaptive) immune response robustness, and if the infection clears, maintenance of antibody production. For persons with long-standing subgingival infection (active periodontitis), high antibody titers may reflect a non-productive or suboptimal immune response (35). However, for others, high antibody titers may reflect an effective immune response that protects them from developing infection or aids in clearing infection, and thus, avoiding periodontitis (36). Thus, participants with detectable antibodies likely are heterogenous, making the interpretation of associations with lung cancer not straightforward. To address this complexity, we explored bacteria counts to antibody ratios, and joint count and antibody categories. Based on the former, we noted that the ratios for *P. intermedia* and *P. gingivalis* were associated with a higher lung cancer risk, suggesting an exhausted or otherwise insufficient antibody response may be etiologically relevant. For *T. forsythensis*, both a high and low ratio (versus middle tertile) was associated with higher lung cancer risk; the latter possibly suggesting that low, persistent colonization that is not cleared by an antibody response is etiologically relevant. Based on joint categories, any combination versus being both ≤LOD for antibodies and not detected for bacterial DNA for *F. nucleatum* was positively associated with lung cancer risk, suggesting the either current or past exposure is etiologically relevant. These analyses were based on a small number of lung cancer cases (N=40), and these interpretations are speculative.

Fifth, we evaluated associations for antibodies to and counts for specific oral bacteria summed based on previously described complexes of subgingival bacteria that co-occur. However, the biology of oral microbiota in periodontal disease etiology may be more complex with disturbances in commensal bacteria along with the presence of pathogenic bacteria being necessary (37). Thus, our approach may not have fully captured the relevant dysbiosis for lung cancer risk. Sixth, we did not include edentulous participants because they were not eligible for the ARIC dental examination. Edentulism can be caused by severe periodontal disease, which was associated with increased lung cancer risk in ARIC. Historically, and still today, in severe, intractable cases, treatment may involve removal of all teeth, which can result in lower oral pathogen burden (38). It is possible that edentulous participants have lower antibody levels while having a history of severe periodontal disease (35). Thus, in this study we cannot determine the generalizability of the findings to edentulous persons. Seventh, we cannot fully rule out residual confounding by smoking

because we were not able to perform the analysis in never-smokers due to few lung cancer cases. Eighth, while the sample size was not large, this study was powered to detect HRs in the moderate to larger range. Finally, antibodies were previously measured in ARIC for their known or suspected roles in the etiology of periodontal disease, and our goal was to investigate oral bacterial infection history as the explanation, in part, for the observed association between periodontal disease and lung cancer in ARIC. Hence, we did not perform multiple testing correction for this candidate approach. For antibodies to 18 bacteria or 3 clusters of bacteria, or for counts for 8 bacteria, none of the tertile HRs was conventionally statistically significant. We performed many other analyses expressing these same antibodies and counts as continuous or in subgroups to explore in detail the same overarching hypothesis; some of these HRs were statistically significant at the conventional level. Thus, chance remains an explanation for these findings.

A major strength of this study is the prospective cohort design. Complementing the antibody analyses, we were able to investigate associations for bacteria counts in persons who did not receive antibiotics in advance of the dental examination in which the subgingival plaque was collected. We adjusted for smoking, a strong confounder. We considered lifecourse SES, and in some analyses, used a propensity score to adjust for lifecourse SES and SES-related factors including access to and uptake of medical and dental care; these factors can produce disparities in both the development of periodontal disease and lung cancer. Lastly, the ARIC study population has similar prevalences of major cancer risk factors and cancer incidence and cancer mortality rates compared to the same age, race, and sex group in the US population over the same period of time (21). Hence, our findings are likely to be generalizable to similar populations in the US.

In summary, this prospective study suggests that orange complex oral bacteria may be associated with an increased lung cancer risk and support the need for more research. Ultimately, if the associations for periodontal disease and periodontal pathogens with lung cancer risk are supported, this may inform novel lung cancer prevention strategies, including access to dental care.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used:

ARIC	Atherosclerosis Risk in Communities
BMI	Body mass index
CI	Confidence interval
HR	Hazard ratio
HRT	Hormone replacement therapy
IQR	Interquartile range
IgG	Immunoglobulin G
LOD	Limit of detection
NSCLC	Non-small cell lung cancer
SES	Socioeconomic status

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Table 1.

Baseline characteristics of participants with antibodies to *P. gingivalis* at or below and above the limit of detection in ARIC [a]

Characteristic	At or Below LOD	Above LOD	*P* Value [c]
Number	2587	1676	
Mean age (SD, y)	62.0(5.6)	62.2 (5.7)	0.37
Female (%)	55.2	54.7	0.71
Black (%)	7.7	29.7	<0.0001
Education level (%)			
<= 11 years (basic)	10.9	16.7	<0.0001
12–16 years (intermediate)	45.0	42.5	
17–21 years (advanced)	44.1	40.8	
Family history of cancer (%)	59.2	56.0	0.046
Mean body mass index [b] (SE, kg/m^2)	29.2 (0.1)	29.6 (0.1)	0.02
Cigarette smoking status [b] (%)			
Never smoker	14.3	12.7	0.06
Former smoker	42.8	46.5	
Current smoker	43.0	40.8	
Mean packyears smoked [b] (SE)	22.7 (0.8)	24.4 (0.8)	0.07
Alcohol drinking status [b] (%)			
Never drinker	43.1	41.4	0.19
Former drinker	38.0	41.1	
Current drinker	18.9	17.5	
Having diagnosed diabetes (%)	9.6	12.4	0.003

[a] Characteristics were measured at ARIC visit 4. Participants attended visit 4 were free of cancer at visit 4; LOD = limit of detection (20 ng/mL).

[b] Body mass index, cigarette smoking status, packyears smoked, and alcohol drinking status were adjusted for age at visit 4 and race.

[c] P value was calculated using the t-test, Pearson's chi-squared test, or Wald test (for adjusted values).

Table 2.

Median [a] (IQR) concentration of pre-diagnostic antibodies to 18 oral bacteria (ng/mL) in lung cancer cases and non-cases in 4263 participants in ARIC [b]

Bacterium	Case (N = 118)	Non-case (N = 4145)	*P* Value [c]
A. actinomycetemcomitans	93.6 (180.4)	72.0 (145.5)	0.07
A. viscosus	10.6 (72.1)	15.9 (76.8)	0.20
C. ochracea	13.8 (42.1)	17.1 (38.6)	0.56
C. rectus	8.2 (33.0)	11.9 (33.2)	0.16
E. corrodens	6.5 (25.5)	7.1 (20.2)	0.89
F. nucleatum	6.2 (19.4)	5.8 (18.0)	0.76
H. pylori	13.6 (59.0)	11.1 (36.7)	0.70
P. gingivalis	15.0 (36.3)	12.5 (44.6)	0.58
P. intermedia	41.0 (101.9)	31.9 (82.7)	0.48
P. micra	74.5 (200.7)	46.3 (102.9)	0.01
P. nigrescens	48.3 (129.9)	58.3 (147.8)	0.38
S. intermedius	12.8 (73.6)	14.6 (63.2)	0.60
S. noxia	5.2 (24.0)	5.0 (19.6)	0.84
S. oralis	26.9 (83.8)	16.4 (54.2)	0.49
S. sanguis	19.9 (85.1)	12.7 (66.0)	0.27
T. denticola	17.9 (28.6)	21.3 (28.7)	0.12
T. forsythensis	22.0 (31.3)	21.0 (40.9)	0.83
V. parvula	3.3 (13.8)	3.9 (13.3)	0.51

[a] Limit of detection (LOD) for antibody concentration is 20 ng/mL.

[b] Lung cancer cases are participants who developed first primary lung cancer during the follow-up; IQR = interquartile range.

[c] P value was calculated with Wilcoxon rank-sum test.

Table 3.

Adjusted hazard ratios of lung cancer incidence by three levels of antibodies to 18 oral bacteria in 4263 participants in ARIC [a]

Bacterium	Lung cancer cases/Person-Years	Median concentration (ng/mL)	Model 1 HR (95% CI) [b]	Model 2 HR (95% CI) [c]	P_{trend} [d]
A. actinomycetemcomitans					
Group 1 (lowest)	20/12587	9.7	1.00 (ref.)	1.00 (ref.)	0.13
Group 2	41/25534	50.6	0.94 (0.55–1.60)	0.94 (0.55–1.58)	
Group 3 (highest)	57/25200	212.7	1.28 (0.76–2.15)	1.28 (0.78–2.12)	
A. viscosus					
Group 1 (lowest)	68/34054	2.3	1.00 (ref.)	1.00 (ref.)	0.89
Group 2	25/14492	41.9	0.89 (0.56–1.40)	0.80 (0.50–1.28)	
Group 3 (highest)	25/14776	211.2	0.84 (0.53–1.34)	0.95 (0.60–1.50)	
C. ochracea					
Group 1 (lowest)	66/34502	5.7	1.00 (ref.)	1.00 (ref.)	0.87
Group 2	24/14609	30.9	0.81 (0.51–1.30)	0.95 (0.60–1.51)	
Group 3 (highest)	28/14210	80.1	0.95 (0.60–1.48)	0.97 (0.62–1.51)	
C. rectus					
Group 1 (lowest)	73/39566	4.5	1.00 (ref.)	1.00 (ref.)	0.34
Group 2	28/12013	30.7	1.16 (0.75–1.81)	1.21 (0.78–1.89)	
Group 3 (highest)	17/11742	87.1	0.70 (0.41–1.20)	0.74 (0.43–1.28)	
E. corrodens					
Group 1 (lowest)	83/47298	3.4	1.00 (ref.)	1.00 (ref.)	0.46
Group 2	15/7918	26.6	1.01 (0.58–1.75)	1.03 (0.58–1.82)	
Group 3 (highest)	20/8104	65.8	1.25 (0.76–2.05)	1.21 (0.74–2.00)	
F. nucleatum					
Group 1 (lowest)	89/48792	3.0	1.00 (ref.)	1.00 (ref.)	0.83
Group 2	13/7287	26.9	0.92 (0.51–1.65)	0.99 (0.55–1.80)	
Group 3 (highest)	16/7243	65.6	1.11 (0.65–1.90)	1.07 (0.62–1.84)	
H. pylori					
Group 1 (lowest)	70/39859	3.3	1.00 (ref.)	1.00 (ref.)	0.48
Group 2	18/11883	32.0	0.79 (0.47–1.32)	0.72 (0.42–1.22)	
Group 3 (highest)	30/11579	118.6	1.39 (0.90–2.16)	1.15 (0.74–1.79)	
P. gingivalis					
Group 1 (lowest)	71/38613	5.7	1.00 (ref.)	1.00 (ref.)	0.50
Group 2	31/12447	35.6	1.29 (0.84–1.98)	1.05 (0.67–1.64)	
Group 3 (highest)	16/12261	193.2	0.67 (0.38–1.18)	0.83 (0.47–1.45)	
P. intermedia					
Group 1 (lowest)	42/25392	6.6	1.00 (ref.)	1.00 (ref.)	0.48
Group 2	37/19038	38.3	1.11 (0.71–1.74)	1.25 (0.80–1.95)	
Group 3 (highest)	39/18892	156.0	1.14 (0.73–1.78)	1.22 (0.78–1.91)	

Bacterium	Lung cancer cases/Person-Years	Median concentration (ng/mL)	Model 1 HR (95% CI) [b]	Model 2 HR (95% CI) [c]	P_{trend} [d]
P. micra					
Group 1 (lowest)	30/18551	7.5	1.00 (ref.)	1.00 (ref.)	0.78
Group 2	32/22603	41.6	0.84 (0.51–1.39)	0.85 (0.52–1.40)	
Group 3 (highest)	56/22167	169.6	1.44 (0.92–2.25)	0.99 (0.63–1.57)	
P. nigrescens					
Group 1 (lowest)	32/15616	8.3	1.00 (ref.)	1.00 (ref.)	0.60
Group 2	42/23977	45.9	0.81 (0.51–1.29)	0.97 (0.61–1.53)	
Group 3 (highest)	44/23728	225.6	0.85 (0.54–1.35)	1.09 (0.69–1.74)	
S. intermedius					
Group 1 (lowest)	66/35425	3.4	1.00 (ref.)	1.00 (ref.)	0.89
Group 2	23/14126	37.5	0.88 (0.55–1.42)	0.99 (0.61–1.61)	
Group 3 (highest)	29/13770	248.5	1.10 (0.71–1.71)	1.03 (0.66–1.61)	
S. noxia					
Group 1 (lowest)	83/47910	2.0	1.00 (ref.)	1.00 (ref.)	0.26
Group 2	23/7511	30.0	1.59 (1.00–2.54)	1.58 (0.98–2.53)	
Group 3 (highest)	12/7901	217.8	0.89 (0.48–1.64)	0.67 (0.34–1.32)	
S. oralis					
Group 1 (lowest)	56/34543	4.1	1.00 (ref.)	1.00 (ref.)	0.21
Group 2	28/14642	35.9	1.18 (0.75–1.86)	1.00 (0.63–1.58)	
Group 3 (highest)	34/14137	149.5	1.44 (0.93–2.21)	1.30 (0.84–2.01)	
S. sanguis					
Group 1 (lowest)	59/36488	2.5	1.00 (ref.)	1.00 (ref.)	0.62
Group 2	30/13288	40.7	1.41 (0.91–2.20)	1.28 (0.82–2.01)	
Group 3 (highest)	29/13545	226.0	1.35 (0.87–2.12)	1.16 (0.73–1.82)	
T. denticola					
Group 1 (lowest)	64/30472	10.9	1.00 (ref.)	1.00 (ref.)	0.35
Group 2	25/16508	27.0	0.68 (0.42–1.07)	0.66 (0.42–1.06)	
Group 3 (highest)	29/16340	60.7	0.79 (0.50–1.22)	0.81 (0.52–1.26)	
T. forsythensis					
Group 1 (lowest)	53/30841	8.7	1.00 (ref.)	1.00 (ref.)	0.68
Group 2	39/16253	29.4	1.33 (0.88–2.02)	1.35 (0.89–2.06)	
Group 3 (highest)	26/16228	87.1	0.90 (0.56–1.44)	1.13 (0.70–1.82)	
V. parvula					
Group 1 (lowest)	97/52851	1.8	1.00 (ref.)	1.00 (ref.)	0.69
Group 2 (highest)	21/10470	36.2	1.01 (0.63–1.63)	0.91 (0.56–1.47)	
Red complex					
Group 1 (lowest)	45/21329	24.8	1.00 (ref.)	1.00 (ref.)	0.22
Group 2	41/21174	71.7	0.88 (0.57–1.34)	1.03 (0.68–1.58)	
Group 3 (highest)	32/20818	233.4	0.68 (0.43–1.08)	0.77 (0.48–1.23)	
Orange complex					

Bacterium	Lung cancer cases/Person-Years	Median concentration (ng/mL)	Model 1 HR (95% CI) [b]	Model 2 HR (95% CI) [c]	P_{trend} [d]
Group 1 (lowest)	32/21572	60.7	1.00 (ref.)	1.00 (ref.)	0.28
Group 2	40/21077	212.6	1.25 (0.78–1.99)	1.32 (0.83–2.10)	
Group 3 (highest)	46/20673	672.5	1.39 (0.88–2.20)	1.36 (0.86–2.14)	
P. intermedia* + *P. nigrescens					
Group 1 (lowest)	40/21550	21.3	1.00 (ref.)	1.00 (ref.)	0.81
Group 2	40/21072	96.5	0.98 (0.63–1.53)	1.18 (0.76–1.83)	
Group 3 (highest)	38/20700	397.8	0.93 (0.59–1.45)	1.11 (0.70–1.74)	
All bacteria					
Group 1 (lowest)	38/21338	224.9	1.00 (ref.)	1.00 (ref.)	0.96
Group 2	39/20976	733.8	1.01 (0.65–1.59)	1.18 (0.76–1.85)	
Group 3 (highest)	41/21008	2143.6	1.04 (0.66–1.62)	1.06 (0.68–1.66)	

[a] For each bacterium, Group 1 was participants with an antibody concentration at or below the limit of detection (20 ng/mL); Group 2 and Group 3 were participants with an antibody concentration above the limit of detection, divided at the median concentration; if the proportion of at or below the limit of detection was larger than 80% for the antibody, only one group was set for those with an antibody level above the limit of detection; For the sum of bacteria (red complex, orange complex, *P. intermedia* + P. *nigrescens*, and all bacteria), participants were divided into tertiles, where Group 1 was the lowest tertile and Group 3 was the highest tertile; All bacteria include 18 bacteria described in the study; Red complex bacteria include *P. gingivalis*, *T. denticola*, and *T. forsythensis*; Orange complex bacteria include *C. rectus*, *F. nucleatum*, *P. intermedia*, *P. micra*, and *P. nigrescens*; HR = hazard ratio; CI = confidence interval; HRT = hormone replacement therapy; BMI = body mass index.

[b] Model 1 was adjusted for age, joint terms for field center and race (Black from Jackson; Black from Forsyth; White from Forsyth; White from Washington County [reference is White from Minneapolis]), and joint terms for sex and HRT use (female user, female nonuser [reference is men]).

[c] Model 2 was adjusted for all the variables in model 1, and additionally adjusted for cigarette smoking status, packyears smoked, alcohol drinking status, BMI, diagnosed diabetes status, undiagnosed diabetes status, at risk for diabetes status, family history of cancer; the model was weighted with a propensity score to control the confounding by socioeconomic status and access to and uptake of medical and dental care.

[d] P value for trend for model 2 was from the Wald test of the coefficient for the ordinal variable of the 3 groups in a Cox proportional hazards regression.

Table 4.

Adjusted hazard ratios of lung cancer for the sum of antibodies to total, red, and orange complex oral bacteria, overall and by follow-up time, sex, and race in 4263 participants in ARIC [a]

Model [b]	HR (per IQR) (95% CI)	*P* Value [c]
Overall		
All bacteria	1.04 (0.89–1.21)	0.61
Red complex bacteria	0.94 (0.82–1.08)	0.41
Orange complex bacteria	**1.15 (1.02–1.29)**	**0.02**
P. intermedia* + *P. nigrescens	**1.15 (1.04–1.26)**	**0.004**
P. intermedia	**1.12 (1.04–1.21)**	**0.005**
P. nigrescens	**1.11 (1.03–1.21)**	**0.01**
Shorter follow-up time [d]		
All bacteria	**1.23 (1.06–1.43)**	**0.01**
Red complex bacteria	0.99 (0.84–1.18)	0.94
Orange complex bacteria	**1.29 (1.12–1.49)**	**0.001**
P. intermedia* + *P. nigrescens	**1.22 (1.10–1.34)**	**0.0001**
P. intermedia	**1.16 (1.07–1.25)**	**0.0003**
P. nigrescens	**1.21 (1.09–1.34)**	**0.0002**
Longer follow-up time [d]		
All bacteria	0.94 (0.73–1.21)	0.62
Red complex bacteria	0.96 (0.81–1.15)	0.67
Orange complex bacteria	1.08 (0.89–1.31)	0.45
P. intermedia + *P. nigrescens*	1.08 (0.91–1.27)	0.38
P. intermedia	1.08 (0.95–1.22)	0.24
P. nigrescens	1.05 (0.89–1.23)	0.58
Female		
All bacteria	0.99 (0.77–1.28)	0.96
Red complex bacteria	0.89 (0.70–1.14)	0.35
Orange complex bacteria	1.03 (0.85–1.27)	0.75
P. intermedia + *P. nigrescens*	1.10 (0.96–1.27)	0.19
P. intermedia	1.08 (0.94–1.23)	0.25
P. nigrescens	1.10 (0.95–1.27)	0.20
Male		
All bacteria	1.08 (0.89–1.31)	0.44
Red complex bacteria	0.99 (0.84–1.17)	0.93
Orange complex bacteria	**1.27 (1.08–1.49)**	**0.004**
P. intermedia* + *P. nigrescens	**1.22 (1.07–1.38)**	**0.003**
P. intermedia	**1.17 (1.06–1.29)**	**0.002**
P. nigrescens	**1.22 (1.04–1.43)**	**0.02**
Black		

Model [b]	HR (per IQR) (95% CI)	*P* Value [c]
All bacteria [e]	1.13 (0.89–1.31)	0.46
Red complex bacteria	0.89 (0.66–1.20)	0.93
Orange complex bacteria	1.29 (0.95–1.73)	0.10
P. intermedia* + *P. nigrescens	**1.22 (1.00–1.48)**	**0.045**
P. intermedia	1.11 (0.96–1.30)	0.17
P. nigrescens	**1.31 (1.05–1.65)**	**0.02**
White		
All bacteria	1.05 (0.88–1.25)	0.58
Red complex bacteria	0.98 (0.85–1.13)	0.93
Orange complex bacteria	1.13 (0.99–1.29)	0.08
P. intermedia* + *P. nigrescens	**1.13 (1.01–1.26)**	**0.003**
P. intermedia	**1.12 (1.02–1.23)**	**0.02**
P. nigrescens	1.11 (0.98–1.25)	0.09

[a] Sum of concentrations entered as a continuous variable in the model; All bacteria: 18 bacteria; Red complex: *P. gingivalis*, *T. denticola*, and *T. forsythensis*; Orange complex: *C. rectus*, *F. nucleatum*, *P. intermedia*, *P. micra*, and *P. nigrescens*; HR = hazard ratio; CI = confidence interval; HRT = hormone replacement therapy; BMI = body mass index.

[b] All models were adjusted for age, joint terms for field center and race (Black from Jackson; Black from Forsyth; White from Forsyth; White from Washington County [reference is White from Minneapolis]), joint terms for sex and HRT use (female user, female nonuser [reference is men]), cigarette smoking status, packyears smoked, alcohol drinking status, BMI, diagnosed diabetes status, undiagnosed diabetes status, at risk for diabetes status, family history of cancer, and lifecourse socioeconomic status.

[c] P value was from the Wald test of the coefficient for the continuous antibody concentration in a Cox proportional hazards regression.

[d] Follow-up time was stratified at the median follow-up time among the cases as follows - shorter: ≤ 9.9 years (814 participants, 59 lung cancer cases), longer: >9.9 years (3,449 participants, 59 lung cancer cases).

[e] The association between the antibody concentration of *P. intermedia* and lung cancer risk in overall population, and the association between the sum of antibody concentration of orange complex bacteria in Black participants differed from linear based on the result of the likelihood ratio test comparing the restricted cubic spline model with the continuous model.

Table 5.

Adjusted hazard ratios of lung cancer incidence by DNA-derived counts for 8 oral bacteria in 1287 participants in ARIC [a]

Bacterium	Cases/ Person-Years	Median DNA-derived bacteria counts	Model 1 HR (95% CI) [b]	Model 2 HR (95% CI) [c]	P_{trend} [d]	Model 3 HR (95% CI) [e]
A. actinomycetemcomitans						
Group 1 (not detected)	5/4707	0	1.00 (ref.)	1.00 (ref.)	0.15	1.00 (ref.)
Group 2	15/7291	1740	1.81 (0.65–5.01)	1.72 (0.62–4.72)		1.99 (0.76–5.19)
Group 3 (highest)	20/6809	9684	2.37 (0.84–6.65)	2.27 (0.84–6.16)		
per IQR			/	0.94 (0.83–1.06)		/
C. rectus						
Group 1 (not detected)	12/5003	0	1.00 (ref.)	1.00 (ref.)	0.73	1.00 (ref.)
Group 2	10/7072	2360	0.58 (0.25–1.35)	0.62 (0.27–1.40)		0.84 (0.41–1.74)
Group 3 (highest)	18/6732	26014	0.95 (0.42–2.16)	0.83 (0.40–1.71)		
per IQR			/	0.94 (0.84–1.04)		/
F. nucleatum						
Group 1 (not detected)	6/5772	0	1.00 (ref.)	1.00 (ref.)	0.26	1.00 (ref.)
Group 2	16/6732	6400	2.2 (0.86–5.63)	2.21 (0.84–5.81)		2.27 (0.93–5.55)
Group 3 (highest)	18/6303	93709	2.36 (0.87–6.38)	2.50 (0.95–6.62)		
per IQR			/	0.99 (0.95–1.03)		/
P. gingivalis						
Group 1 (not detected)	8/5469	0	1.00 (ref.)	1.00 (ref.)	0.96	1.00 (ref.)
Group 2	17/6840	1089	1.71 (0.74–3.96)	1.94 (0.81–4.61)		1.61 (0.73–3.56)
Group 3 (highest)	15/6499	5696	1.39 (0.57–3.36)	1.47 (0.62–3.52)		
per IQR			/	1.00 (0.99–1.01)		/
P. intermedia						
Group 1 (not detected)	10/6424	0	1.00 (ref.)	1.00 (ref.)	0.36	1.00 (ref.)
Group 2	14/6266	5491	1.37 (0.61–3.1)	1.37 (0.60–3.15)		1.42 (0.67–3.02)
Group 3 (highest)	16/6118	36965	1.49 (0.65–3.43)	1.60 (0.69–3.74)		
per IQR			/	0.98 (0.93–1.04)		/
P. nigrescens						
Group 1 (not detected)	11/5638	0	1.00 (ref.)	1.00 (ref.)	0.75	1.00 (ref.)
Group 2	14/6651	3348	1.06 (0.48–2.34)	1.25 (0.56–2.76)		1.03 (0.51–2.10)
Group 3 (highest)	15/6518	30669	1.00 (0.45–2.26)	1.03 (0.46–2.32)		
per IQR			/	0.99 (0.94–1.04)		/
T. denticola						
Group 1 (not detected)	13/5465	0	1.00 (ref.)	1.00 (ref.)	0.66	1.00 (ref.)
Group 2	9/6932	2778	0.52 (0.22–1.23)	0.49 (0.20–1.16)		0.62 (0.31–1.27)
Group 3 (highest)	18/6411	20224	1.04 (0.46–2.37)	0.96 (0.42–2.18)		
per IQR			/	1.00 (0.98–1.02)		/

Bacterium	Cases/ Person-Years	Median DNA-derived bacteria counts	Model 1 HR (95% CI) [b]	Model 2 HR (95% CI) [c]	P_{trend} [d]	Model 3 HR (95% CI) [e]
T. forsythensis						
Group 1 (not detected)	14/5213	0	1.00 (ref.)	1.00 (ref.)	0.79	1.00 (ref.)
Group 2	8/7015	1207	0.40 (0.16–0.95)	**0.37 (0.15–0.89)**		0.56 (0.28–1.13)
Group 3 (highest)	18/6580	10328	0.81 (0.36–1.79)	0.75 (0.36–1.59)		
per IQR			/	0.99 (0.96–1.03)		/
Red complex						
Group 1 (lowest)	14/6298	1485	1.00 (ref.)	1.00 (ref.)	0.47	1.00 (ref.)
Group 2	8/6498	7097	0.52 (0.22–1.27)	0.48 (0.20–1.17)		0.72 (0.36–1.44)
Group 3 (highest)	18/6012	40858	1.14 (0.49–2.61)	1.05 (0.49–2.27)		
per IQR			/	1.00 (0.98–1.02)		/
Orange complex						
Group 1 (lowest)	10/6293	5371	1.00 (ref.)	1.00 (ref.)	0.54	1.00 (ref.)
Group 2	14/6445	25745	1.27 (0.56–2.87)	1.08 (0.49–2.38)		1.28 (0.61–2.69)
Group 3 (highest)	16/6069	204480	1.42 (0.59–3.42)	1.33 (0.60–2.96)		
per IQR			/	0.98 (0.93–1.04)		/
P. intermedia* + *P. nigrescens						
Group 1 (lowest)	11/6291	978	1.00 (ref.)	1.00 (ref.)	0.59	1.00 (ref.)
Group 2	13/6338	10715	1.18 (0.53–2.65)	0.85 (0.38–1.87)		1.09 (0.53–2.23)
Group 3 (highest)	16/6179	74542	1.29 (0.57–2.91)	1.04 (0.47–2.26)		
per IQR			/	0.99 (0.94–1.03)		/
All bacteria						
Group 1 (lowest)	10/6309	1051	1.00 (ref.)	1.00 (ref.)	0.61	1.00 (ref.)
Group 2	14/6449	35746	1.27 (0.56–2.89)	0.98 (0.43–2.24)		1.13 (0.53–2.42)
Group 3 (highest)	16/6050	283846	1.40 (0.57–3.41)	1.34 (0.58–3.11)		
per IQR			/	0.98 (0.93–1.04)		/

[a] For each bacterium, Group 1 was participants who did not have that bacterium detected (count=0: *P. gingivalis* 28.7%, *P. intermedia* 34.1%, *P. nigrescens* 29.0%, *T. forsythensis* 27.7%, *T. denticola* 29.1%, *A. actinomycetemcomitans* 24.0%, *C. rectus* 26.0%, *F. nucleatum* 30.6%); Group 2 and Group 3 were participants who had that bacterium detected divided at the median count. For the sum of bacteria (red complex, orange complex, *P. intermedia* + *P. nigrescens*, and all bacteria), participants were divided into tertiles, where Group 1 was the lowest tertile and Group 3 was the highest tertile; All bacteria include 8 bacteria described in the bacteria count part in the study; Red complex bacteria include *P. gingivalis*, *T. denticola*, and *T. forsythensis*; Orange complex bacteria include *C. rectus*, *F. nucleatum*, *P. intermedia*, and *P. nigrescens*; HR = hazard ratio; CI = confidence interval; IQR = interquartile range; HRT = hormone replacement therapy; BMI = body mass index.

[b] Model 1 was adjusted for age, joint terms for field center and race (Black from Jackson; Black from Forsyth; White from Forsyth; White from Washington County [reference is White from Minneapolis]), and joint terms for sex and HRT use (female user, female nonuser [reference is men]).

[c] Model 2 was adjusted for all the variables in model 1, and additionally adjusted for cigarette smoking status, packyears smoked, alcohol drinking status, BMI, diagnosed diabetes status, undiagnosed diabetes status, at risk for diabetes status, family history of cancer; the model was weighted with a propensity score to control the confounding by socioeconomic status and access to and uptake of medical and dental care.

[d] P value for trend for model 2 was from the Wald test of the coefficient for the ordinal variable of the 3 groups in a Cox proportional hazards regression

[e] Model 3 compared participants in Group 2 and Group 3 jointly with Group 1; Model 3 was adjusted for all the variables in Model 2.