ORIGINAL REPORT: EPIDEMIOLOGIC RESEARCH

Serum IgG Antibodies against Periodontal Microbes and Cancer Mortality

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Abstract: ***Introduction:*** *Periodontitis is a chronic inflammatory condition initiated by microorganisms and is positively linked to systemic conditions such as cancer, cardiovascular disease, and diabetes mellitus.*

Objectives: *To prospectively investigate associations between empirically derived clusters of IgG antibodies against 19 selected periodontal microorganisms and cancer mortality in a representative sample of the US population.*

Methods: *We evaluated 6,491 participants aged ≥40 y from the Third National Health and Nutrition Examination Survey (1988 to 1994), who had complete data on IgG antibody titers against 19 selected periodontal microorganisms and were free of cardiovascular disease and cancer. In a prior study, antibodies were categorized into 4 mutually exclusive groups via cluster analysis: red-green, orange-red, yellow-orange, and orange-blue. Cluster scores were estimated by summing z scores of*

the antibody titers making up each cluster. Participants were followed up to death until December 31, 2011. Cox proportional hazard models were applied to estimate hazard ratios (HRs) and 95% CIs for all-cancer mortality by tertiles of cluster scores.

Results: *During follow-up for a median of 15.9 y, there were 2,702 deaths (31.3%), including 631 cancer-related deaths (8.1%). After adjusting for multiple confounders, the orange-blue cluster was inversely associated with cancer mortality (tertile 2 vs. tertile 1: HR = 0.67, 95% CI = 0.54 to 0.84; tertile 3 vs tertile 1: HR = 0.62, 95% CI = 0.46 to 0.84). The association between the yellow-orange cluster and all-cancer mortality was also inverse but not significant, and the orange-red cluster and the red-green cluster were not associated with all-cancer mortality.*

Conclusions: *Antibodies against* Eubacterium nodatum *and* Actinomyces naeslundii *may be novel predictors of cancer mortality.*

If further studies establish a causal relationship between these antibodies and cancer mortality, they could be targets to prevent possible systemic effects of periodontal disease with potential interventions to raise their levels.

Knowledge Transfer Statement:

Periodontal antibodies against Eubacterium nodatum *and* Actinomyces naeslundii *were inversely associated with cancer mortality among adults followed up for an average of 16 y. Periodontal antibodies may predict cancer mortality.*

Keywords: bacterial antibodies, periodontal diseases, neoplasms, mortality rate, cluster analysis, oral health

Introduction

Inflammation is a key mediator of overall cancer risk (Heikkila et al. 2018). As periodontal disease is a low-grade chronic infection of supporting tooth structures leading to systemic

inflammation and affecting almost half of all US adults, it is plausible that periodontal disease could play a role in cancer pathology (Eke et al. 2012). Emerging evidence from epidemiologic studies has linked periodontal disease with various types of cancer, including cancer of the oral cavity and other sites (Fitzpatrick and Katz 2010; Ahn, Segers, and Hayes 2012; Michaud et al. 2013; Zeng et al. 2013; Heikkila et al. 2018). Higher oral cancer risk was observed for individuals with periodontitis as compared with those with gingivitis, further suggesting that periodontal infection may affect cancer risk (Wen et al. 2014). Individuals with periodontal disease receiving treatment had lower cancer risk versus those not receiving treatment in a large population-based cohort in Taiwan (Hwang et al. 2014).

The observed associations between periodontal disease and cancer are suspected to have a microbial basis. Individuals with oral cancer show altered composition of oral microorganisms (Mager et al. 2005), which are suspected to initiate the carcinogenesis. A recent study reported that carriage of periodontal pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were related to increased risk of pancreatic cancer (Fan et al. 2016). Serum IgG antibodies, which are induced by periodontal microorganisms, can remain elevated for up to 15 y (Papapanou et al. 2004) and may thus provide long-lasting protection against subsequent periodontal diseases (Papapanou et al. 2004; Rams et al. 2006) and be a surrogate marker for clinical periodontal status in epidemiologic studies (Papapanou et al. 2001). A twofold increased risk of pancreatic cancer was detected in individuals with greater serum *P. gingivalis* IgG in a large European prospective cohort study (Michaud et al. 2013). Similarly, high levels of antibody against *P. gingivalis* tended to be associated with higher orodigestive cancer mortality (Ahn, Segers, and Hayes 2012). Although there is evidence linking periodontal

disease with cancer risk, the underlying mechanisms are uncertain. As the mouth harbors approximately 700 microorganisms, identifying relevant serum IgG antibodies that can serve as markers is challenging (Schenkein et al. 1993).

The Centers for Disease Control and Prevention released data for antibodies against 19 periodontal microorganisms using stored blood samples from participants of the Third National Health and Nutrition Examination Survey (NHANES III) in 2008 (Vlachojannis et al. 2010). In a prior analysis, we categorized these antibodies into 4 groups via cluster analysis, an empirical approach giving mutually exclusive groups reflecting the way the antibodies grouped together in vivo (Merchant et al. 2014). Specific groups of antibodies defined this way were positively or negatively associated with hyperglycemia, metabolic syndrome, and cardiovascular risk factors (Merchant et al. 2014). These clusters were found to modify the associations between periodontal disease and alcohol intake and physical activity (Merchant et al. 2016; Anderson et al. 2018). The advantages of this approach to examine the relation between periodontal antibodies and systemic outcomes were that type 1 error was minimized and the groups reflected the way that the antibodies clustered in vivo. The aim of this study was to prospectively investigate the underlying associations between these serum IgG antibody clusters and cancer mortality with the nationally representative NHANES III data.

Methods

Data Source

The NHANES III was conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention from 1988 to 1994 among a representative sample of the noninstitutionalized civilian US population. Data were collected via household interview, medical and dental examination, and laboratory tests in

a standardized way with strict quality control procedures (Centers for Disease Control and Prevention 1992; National Center for Health Statistics 1994).

Population Description

The CDC used stored serum from NHANES III participants ≥40 y old at the time of examination to test for IgG antibodies (gravimetric units) against 19 oral bacterial species related to periodontal disease via the "checkerboard" immunoassay technique at the Columbia University College of Dental Medicine. Details are described in NHANES III documentation (Centers for Disease Control and Prevention 2008). Complete IgG data were available for 8,153 individuals. After exclusion of individuals with a self-reported history of CVD or cancer, 6,491 participants were left in the final sample. All participants were followed up for mortality status until December 31, 2011 (National Center for Health Statistics 2013).

Exposure Measures

Cluster Formation and Naming the Clusters

Data from NHANES III participants with data on IgG antibodies were used to derive antibody clusters in a prior study (Merchant et al. 2014). Briefly, serum IgG antibody titers against periodontal microorganisms were grouped into 4 mutually distinct groups via cluster analysis. To name these clusters, we adapted the nomenclature used by Socransky and Haffajee to describe periodontal microorganism groups related to clinical periodontal conditions. They classified the microorganisms into complexes, which they named using the following color scheme: organisms related to periodontal disease (red and orange complexes), to a healthy periodontal state (yellow and purple complexes), to healthy and diseased states (blue complex), and weakly to periodontal disease (green complex; Socransky and Haffajee 2002, 2005).

Antibody clusters were named in the following way. Cluster 1 contained antibodies against 4 organisms, of

which 3 were from the orange complex and 1 from the red complex, and was named the orange-red cluster (*Prevotella melaninogenica, Prevotella intermedia, Prevotella nigrescens, P. gingivalis*). The red-green (*Tannerella forsythia, Treponema denticola, A. actinomycetemcomitans, Eikenella corrodens, Selenomonas noxia, Veillonella parvula, Campylobacter rectus*), yellow-orange (*Streptococcus intermedius, Streptococcus oralis, Streptococcus mutans, Fusobacterium nucleatum, Parvimonas micra, Capnocytophaga ochracea*), and orange-blue (*Eubacterium nodatum, Actinomyces naeslundii*) clusters were similarly named.

Calculating Cluster Score

In the present analyses, we computed z scores for all 19 antibody titers for each individual. Next, we summed z scores for the antibody titers making up each cluster to get a cluster score for each participant. For example, the orange-blue cluster consisted of antibody titers against *E. nodatum* and *A. naeslundii;* z scores of these 2 antibody titers were summed to obtain a score for that cluster, and so on (Merchant et al. 2014).

Outcome Measures

Data on total cancer mortality were abstracted from the NHANES III Linked Mortality File, which contains mortality data for all eligible NHANES III participants. Follow-up started from the date of survey participation and lasted through December 31, 2011, or the date of death, whichever occurred first; the average length of follow-up period in our study was 15.9 y. Underlying cause of death was obtained from the death certificate. According to the National Center for Health Statistics, 96.1% of decedents and 99.4% of living participants were correctly classified with the probabilistic matching algorithm (Brown et al. 2017). Details can be found elsewhere (National Center for Health Statistics 2013).

Covariates

The following covariables were adjusted for in this study to eliminate confounding influences: age, sex, race, educational level, income, smoking status, drinking status, body mass index, diabetes, hypertension, and annual dentist visits. Age at baseline was self-reported and categorized into 2 groups, representing middle-aged people (40 to 64 y) and elderly people (≥65 y). Data on sex (male and female) and race (black, white, and other) were based on self-report. Poverty income ratio and education level, as indicators of socioeconomic status, were divided into 3 groups (≤1.3, >1.3 to ≤3.5, >3.5) and 2 groups (<12 y, ≥12 y completed education), respectively. Smoking status (current, former, never smoker) and drinking status (drinker and nondrinker) were also self-reported. Body mass index was calculated as weight divided by height squared and categorized into normal (≤24.9 kg/m^2), overweight (25 to ≤29.9 kg/m^2), and obese (≥30 kg/m^2). History of diabetes and hypertension were captured according to whether the participant reported being diagnosed with these conditions by a doctor. Annual visit to a dentist was a binary variable. Oral health measures were obtained from the oral examination component of the NHANES III (Centers for Disease Control and Prevention 1992).

Statistical Analysis

SAS 9.4 (SAS Institute) was used to manage and analyze the data. SAS survey procedures used mobile examination center 6-y weights, with weights recommended for strata and primary sampling unit for the NHANES III, to account for the multistage clustered sampling design, unequal probability of selection, missing data, and poststratification to the US population and to get accurate variance estimates. Failure time was defined as the time from examination at the mobile examination center to death from any

type of cancer, or December 31, 2011, whichever occurred first; survival time was the duration from the start of follow-up to the failure time. The level of significance was set as 0.05.

Basic descriptive statistics were calculated for covariates across extreme tertiles of cluster scores. Specifically, for categorical variables, total original number and weighted percentage across categories were presented; for continuous variables, the means and standard errors were calculated. Kaplan-Meier survival curves, the standard method to describe and compare graphically the overall survival, were plotted by 3 tertiles of different cluster scores.

Cox proportional hazard models were run to estimate the hazard ratio and 95% CI, with survival time to cancer mortality as the outcome and with tertiles of scores for orange-red, red-green, yellow-orange, and orange-blue clusters as the exposures. First, we modeled the crude model with only 4 variables representing the tertiles of 4 clusters; then, we adjusted for the sociodemographic variables, including sex, race, and age group; third, we fully adjusted with education level, income (poverty income ratio), smoking, drinking, body mass index, diabetes status, history of hypertension, and annual dentist visits as other covariates. Variables to control for confounding were chosen per prior studies. During model building, estimates were examined for evidence of collinearity while adding variables. To further evaluate potential confounding that could affect the association between periodontal disease and cancer, we conducted subgroup analyses by sex, age group, race, smoking status, and periodontal status. As males and older individuals have a higher cancer risk, we evaluated the associations in joint subgroups of age (40 to 64 vs. ≥65 y) by sex. The proportional hazards assumption was tested by Schoenfeld residuals to ensure appropriate model fit. The study was exempted from full Institutional Review Board review. The

manuscript is in compliance with the STROBE statement.

Results

The cohort of 6,491 individuals was followed up to 23 y (mean, 19.0 y), during which there were 2,702 deaths (31.3%), including 631 cancer-related deaths (8.1%). At the start of follow-up, the individuals were aged 40 to 90 y (median, 52.15 y); 45% were male; and 86% and 10% were White and Black, respectively.

Table 1 describes the demographic and clinical characteristics of the participants by antibody score tertiles. Individuals in the top tertiles of the red-green and orange-red clusters were more likely to be Black, male, and never smokers as compared with those in the bottom tertiles. People in the top tertiles of the yellow-orange and orange-blue clusters were more likely to be younger, nonsmokers, and with higher education and income levels as compared with those in lower tertiles. The unadjusted Kaplan-Meier survival curves for all-cancer mortality by tertiles of each cluster showed that the overall survival probability was >80% over 15.9 y (Fig. 1). Table 2 shows the hazards ratios and 95% CIs from Cox proportional hazard models. The orange-red cluster and the red-green cluster were not associated with all-cancer mortality. The orange-blue cluster was consistently inversely associated with all-cancer mortality. The associations persisted in fully adjusted models; the hazards ratios (95% CIs) were as follows: 0.67 (0.54 to 0.84), tertile 2 versus tertile 1; 0.62 (0.46 to 0.84), tertile 3 versus tertile 1. The association between the yellow-orange cluster and all-cancer mortality was also inverse, but the 95% CIs included 1.

Subgroup analyses (Fig. 2) show results for cancer mortality with forest plots of hazards ratios and 95% CIs from fully adjusted models for each cluster level (lowest tertile reference). Though the trend of the results in the subgroups were similar to the overall findings, associations appeared stronger

for middle-aged individuals, those with periodontal disease, Whites, ever and never smokers, and females. In the joint strata of age (40 to 64 vs. ≥65 y) by sex, the associations were qualitatively similar but stronger among the females and the younger age group (Appendix Fig.).

Discussion

In the present study, we related empirically derived IgG antibody clusters against specific periodontal microorganisms with overall cancer mortality. In multivariable models, individuals with higher levels of orange-blue cluster scores were associated with lower cancer mortality, while the other 3 clusters (yellow-orange, red-green, and orange-red) were not related with cancer mortality. Consistent results were also observed for middle-aged people, Whites, females, and noncurrent smokers. The qualitatively similar but stronger associations among females and younger individuals in the joint strata of age and sex were likely driven by higher sample sizes in these groups.

The orange-blue cluster consisted of antibodies against *E. nodatum* and *A. naeslundii*. *A. naeslundii* has been linked to good oral health (Dewhirst et al. 2010; Desvarieux et al. 2013), with higher counts of this species being found in individuals without periodontal disease as compared with those with periodontitis (Papapanou et al. 2000). Although higher levels of the *E. nodatum* microorganism are detected in individuals with periodontal disease (Haffajee et al. 2006), higher antibody titers against *E. nodatum* have been found in individuals without periodontal disease (Socransky et al. 1998; Papapanou et al. 2000). Although this is a novel finding, the orange-blue cluster defined in the same way has been inversely related with other systemic conditions, such as hyperglycemia and elevated fasting glucose (Merchant et al. 2014; Shrestha et al. 2015), suggesting that the orange-blue cluster may represent a protective factor in the development of subsequent systemic

diseases. The causal role of the orange-blue antibodies in the prevention of periodontal disease and its possible systemic effects needs to be established in future studies, and if the relation is determined to be causal, these antibodies could be a potentially novel target for intervention to improve oral and systemic health.

A few studies have related periodontal microorganisms to risks of developing cancer, indicating a microbial basis of the association between periodontal disease and cancer. Although we did not observe a significant association between the orange-red cluster and cancer mortality, elevated serum IgG antibody against *P. gingivalis*, a widely recognized periodontal pathogen belonging to this cluster, has been associated with higher risk of orodigestive cancer independent of periodontal disease (Ahn, Segers, and Hayes 2012). Similarly, results from a large European nested case-control study indicated that individuals with higher antibody titers against *P. gingivalis* had a twofold increased risk of developing pancreatic cancer, while higher antibodies to commensal oral bacteria were linked to a 45% lower risk of pancreatic cancer (Michaud et al. 2013), which is similar to our finding. Heavier loads of oral pathogens *P. gingivalis* and *A. actinomycetemcomitans* were related with an increased risk of pancreatic cancer in prospective cohort studies, pointing to a potential role of periodontal microorganisms in carcinogenesis, while an inverse association was observed between specific commensal microorganisms (phylum Fusobacteria and its genus *Leptotrichia*) and a decreased pancreatic cancer risk (Fan et al. 2016). Data derived from the same study also indicated that a greater abundance of genera *Corynebacterium* and *Kingella*, both of which are commensal microorganisms in the oral cavity, was associated with a decreased risk of head and neck squamous cell cancer (Hayes et al. 2018). It is thus plausible that individuals with oral microbial stability, periodontal health, and a strong immune

Table 1.

Descriptive Characteristics (Weighted) of Study Population.

	Participants, n^a	Red-Green		Orange-Red	
		Tertile 1	Tertile 3	Tertile 1	Tertile 3
Cluster score range	6,491	−36.09 to −0.38	2.34 to 13.21	−27.28 to −0.65	1.27 to 10.76
Cancer mortality	631	9.70	7.72[b]	9.02	7.39
Age, y					
Middle-aged, 40 to 64	4,441	77.52	78.03	77.63	78.96
Elderly, ≥65	2,050	22.48	21.97	22.37	21.04
Race					
White	4,568	90.40	81.83[b]	91.62	77.82[b]
Black	1,719	7.85	12.55	6.43	15.56
Other	204	1.75	5.63	1.96	6.62
Sex					
Male	2,942	41.30	49.25[b]	39.98	49.07[b]
Female	3,549	58.70	50.75	60.02	50.93
Educational level, y					
<12	2,952	28.95	26.56	27.13	30.24
≥12	3,493	71.05	73.44	72.87	69.76
Income (poverty income)					
Low	1,717	14.25	12.51	13.59	14.57
Middle	3,185	49.41	45.66	44.71	47.26
High	1,589	36.34	41.82	41.70	38.17
Smoking status					
Current	1,518	29.21	17.19[b]	28.63	18.43[b]
Ever smoker	1,945	32.43	34.56	30.52	32.97
Never smoker	3,028	38.36	48.24	40.85	48.60
Drinking status					
Drinker	2,789	50.67	48.24	50.95	50.79
Nondrinker	3,701	49.33	51.76	49.05	49.21
Body mass index, kg/m^2					
Normal, <25	2,095	38.59	33.08	37.88	32.89
Overweight, 25 to 29.9	2,481	35.81	38.17	38.23	36.90
Obesity, ≥30	1,903	25.60	28.76	23.89	30.21
Diabetes status					
Absent	5,797	93.32	94.09	94.48	92.85
Present	687	6.68	5.91	5.52	7.15
History of hypertension					
Absent	4,211	68.13	68.63	69.25	66.14[b]
Present	2,251	31.87	31.37	30.75	33.86
Annual dentist visits					
No	3,806	49.54	48.71	47.24	47.99
Yes	2,476	50.46	51.29	52.76	52.01

(continued)

Table 1.
(continued)

	Participants, n[a]	Yellow-Orange		Orange-Blue	
		Tertile 1	Tertile 3	Tertile 1	Tertile 3
Cluster score range	6,491	−30.20 to −0.26	1.92 to 11.08	−11.72 to −0.45	0.80 to 4.89
Cancer mortality	631	9.91	6.90[b]	11.43	6.05[b]
Age, y					
Middle-aged, 40 to 64	4,441	74.51	79.79[b]	73.09	83.45[b]
Elderly, ≥65	2,050	25.49	20.21	26.91	16.55
Race					
White	4,568	89.00	81.53 [b]	84.63	87.58
Black	1,719	8.51	12.82	11.55	9.20
Other	204	2.49	5.65	3.82	3.22
Sex					
Male	2,942	42.44	44.81[b]	46.16	45.83
Female	3,549	57.56	55.19	53.84	54.17
Educational level, y					
<12	2,952	30.80	27.33[b]	31.65	21.42[b]
≥12	3,493	69.20	72.67	68.35	78.58
Income (poverty income)					
Low	1,717	16.44	13.00[b]	17.78	10.17[b]
Middle	3,185	47.28	47.45	49.01	43.28
High	1,589	36.28	39.55	33.21	46.56
Smoking status					
Current	1,518	29.15	18.33 [b]	30.06	17.65 [b]
Ever smoker	1,945	31.39	33.26	30.99	33.69
Never smoker	3,028	39.46	48.41	38.95	48.65
Drinking status					
Drinker	2,789	49.76	49.27	46.74	53.40 [b]
Nondrinker	3,701	50.24	50.73	53.26	46.60
Body mass index, kg/m^2					
Normal, <25	2,095	39.84	34.01	37.62	34.12
Overweight, 25 to 29.9	2,481	35.35	37.74	37.46	38.23
Obesity, ≥30	1,903	24.81	28.25	24.92	27.65
Diabetes status					
Absent	5,797	92.66	93.62	91.81	95.41
Present	687	7.34	6.38	8.19	4.59
History of hypertension					
Absent	4,211	68.67	69.05	65.94	72.18[b]
Present	2,251	31.33	30.95	34.06	27.82
Annual dentist visits					
No	3,806	50.56	45.45[b]	52.04	44.12[b]
Yes	2,476	49.44	54.55	47.96	55.88

Unless indicated otherwise, all values are expressed as weighted percentages.
[a]Values are unweighted; total numbers may be different as result of missing data.
[b]$P < 0.05$, indicating differences across tertiles for each cluster.

Figure 1. Kaplan-Meier survival curves for cancer mortality by tertiles of 4 cluster scores.
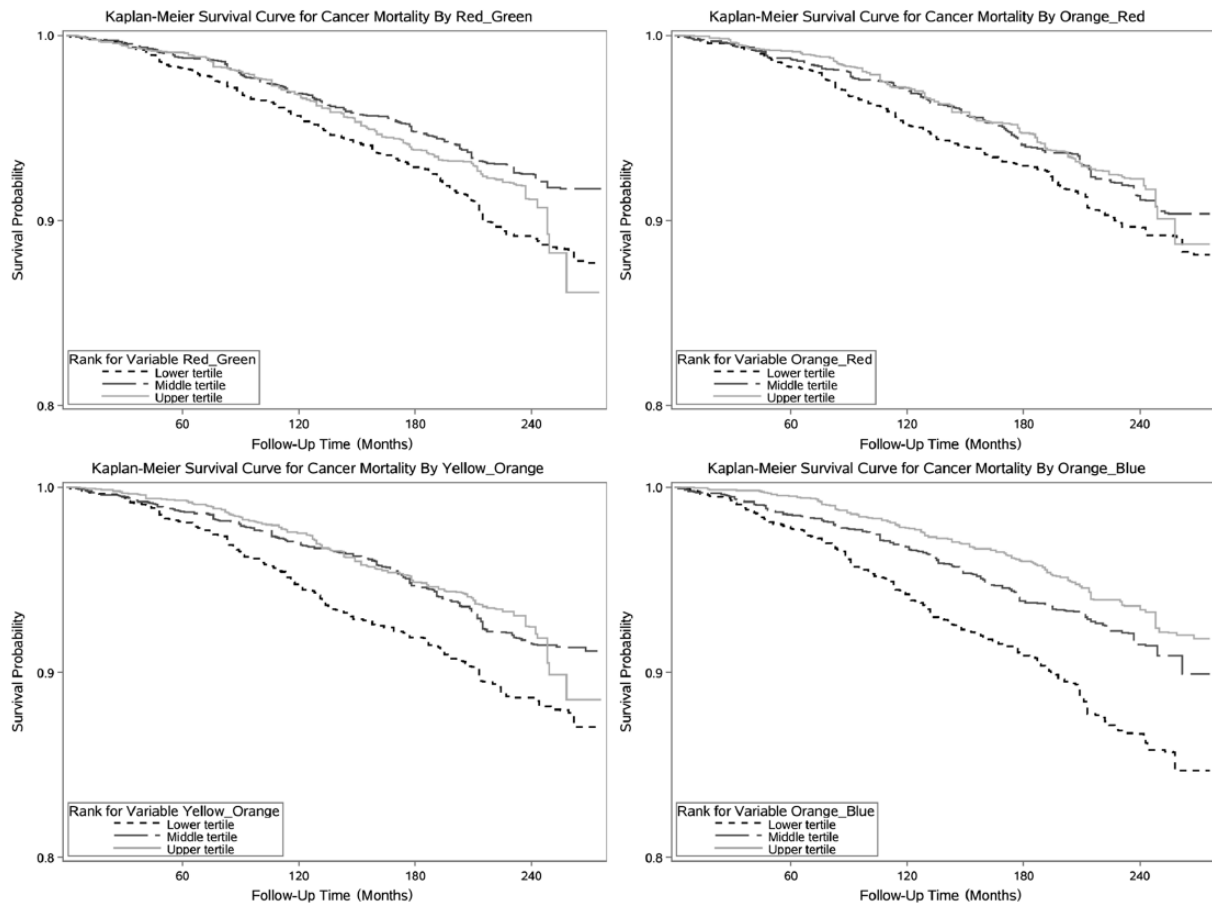

response toward microorganisms may be less likely to develop cancer.

Although certain microorganisms and antibodies have been linked to periodontal disease and various subsequent systemic conditions, the role of periodontal microorganisms in carcinogenesis is not completely understood. However, several plausible hypotheses have been proposed to explain the association. Immune response is a plausible mediator in the causal pathway. It is estimated that infection-driven inflammations are involved in the pathogenesis of 15% of human tumors (Heikkila et al. 2018), while a great many studies report that periodontal disease leads to elevated levels of inflammatory markers, such as CRP and TNF-alpha, which were associated with various systemic conditions, including carcinogenesis (Meyer et al. 2008; Demmer et al. 2012).

In addition, microorganisms associated with periodontal disease have been identified at distant sites, including plaque in the intimal wall of blood vessels and pancreas duct (Kozarov et al. 2005; Swidsinski et al. 2005). In a review of randomized controlled trials, periodontal treatment tended to reduce oral and systemic inflammation and counts of periodontal microorganisms (Merchant and Virani 2017). However, in poor oral health states, specific periodontal bacteria species have the capacity to produce nitrosamines and acetaldehyde; both chemicals are recognized human carcinogens that have been linked to oral cancer and certain upper gastrointestinal cancer types. Thus, increased exposure to these 2 carcinogenic metabolic by-products can result in a higher risk of developing cancer (Fitzpatrick and Katz 2010; Ahn, Chen, and Hayes 2012).

The study had several strengths. It was conducted in a representative US population; data collection was rigorous; and physical examinations and laboratory tests strictly adhered to standardized protocols to minimize measurement errors. In addition, it had a prospective design with a median follow-up of 15.91 y. Serum samples were collected before cancer diagnosis, making it possible to observe the temporal association between exposure variables and subsequent cancer risk; outcome ascertainment was valid and almost complete. Another advantage was the application of cluster analysis. The oral microbiome is complex, consisting of hundreds of microorganisms (Schenkein et al. 1993), which, if evaluated one at a time, would increase the chances of type 1 error because of multiple testing. Antibodies against 19 oral microorganisms that were

Figure 2. Adjusted hazard ratios (95% CIs) of cancer mortality according to tertiles of 4 cluster scores by subgroups of age, race, sex, periodontal status, and smoking status. HR, hazard ratio.
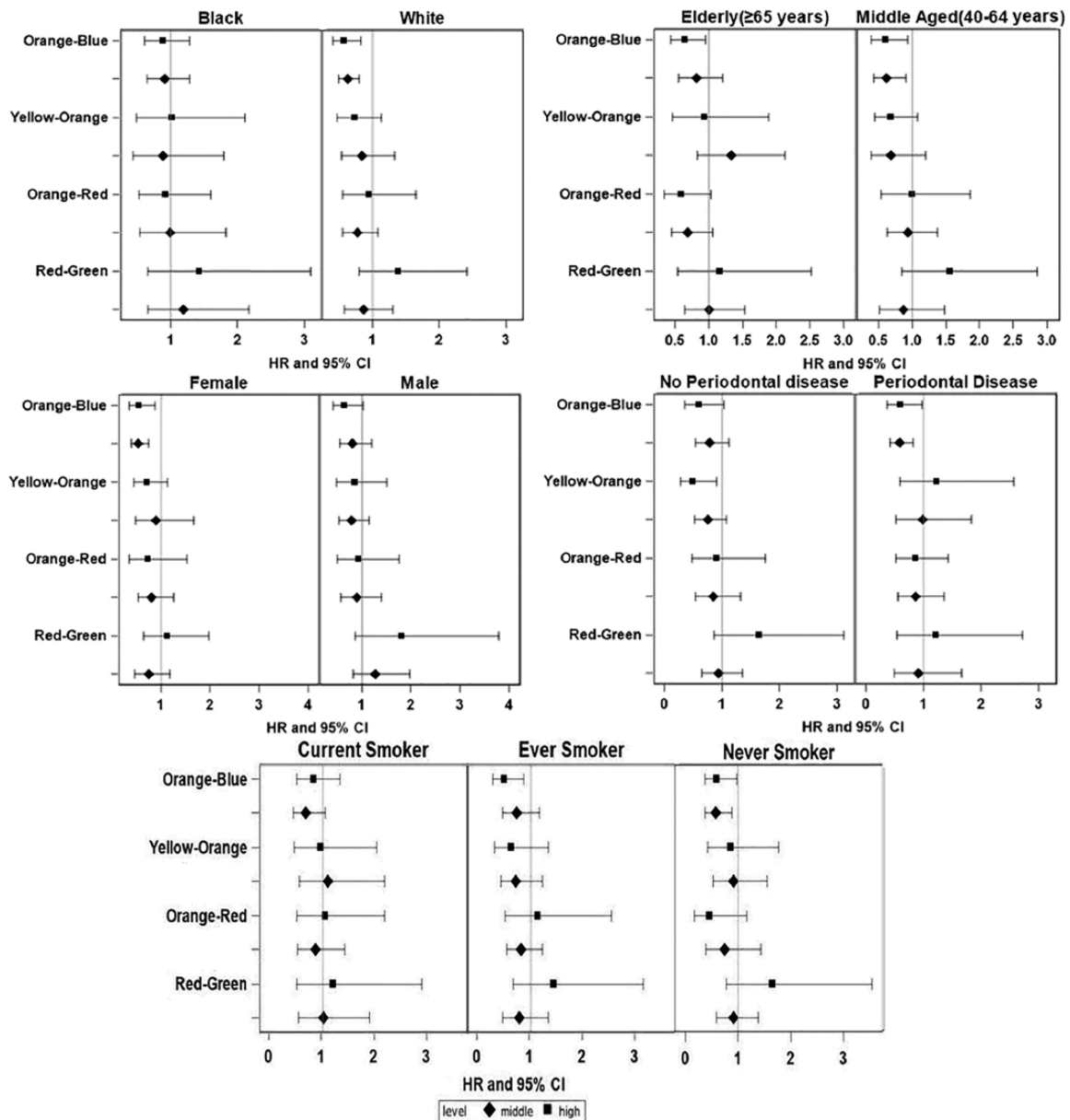
well studied in relation to periodontal disease were assayed in NHANES III. The chances of false-positive findings were further reduced by grouping these antibodies with cluster analysis to define the groups as captured via their natural grouping in vivo. Finally, the association persisted in subgroups, including among never smokers.

However, our study has some limitations. First, antibodies against just 19 periodontal microorganisms were assessed in NHANES III. It is possible that the unmeasured antibodies were

causal and were correlated with the 19 measured antibodies. Second, small numbers of cases limited our ability to evaluate the risk for specific cancers. Given strong associations between periodontitis and cancer type, particularly oral and pancreatic cancer, further studies are required to investigate these associations. The observed association may be underestimated because the outcome was cancer mortality rather than incidence. Increased screening and emerging treatments have increased survival from common cancers, such

as those of the breast and colon, but similar trends have not been reported for pancreatic and oral cancers, which have been related to periodontal disease (Torre et al. 2016; Siegel et al. 2017). Third, we did not explicitly characterize the relation among antibodies against periodontal microorganisms, periodontal disease, and mortality. It is also possible that antibodies making up the groups had opposing actions, which could have attenuated the associations. However, in subgroup analyses, the associations between antibody clusters and mortality were

Table 2.

Cancer Mortality in Relation to 4 Cluster Scores of Serum IgG Antibody Titers against 19 Periodontal Microorganisms.

	Cancer Mortality: HR (95% CI)[a]		
Cluster: Level[b]	**Model 1**	**Model 2**	**Model 3**
Red-green			
Middle	0.95 (0.65 to 1.40)	0.90 (0.62 to 1.31)	0.93 (0.65 to 1.33)
High	1.47 (0.88 to 2.44)	1.32 (0.81 to 2.15)	1.41 (0.86 to 2.29)
Orange-red			
Middle	0.91 (0.68 to 1.22)	0.85 (0.63 to 1.14)	0.83 (0.62 to 1.12)
High	0.94 (0.58 to 1.51)	0.82 (0.51 to 1.32)	0.86 (0.54 to 1.37)
Yellow-orange			
Middle	0.78 (0.52 to 1.18)	0.87 (0.58 to 1.30)	0.86 (0.58 to 1.26)
High	0.70 (0.49 to 1.00)[c]	0.80 (0.55 to 1.18)	0.80 (0.56 to 1.13)
Orange-blue			
Middle	0.62 (0.50 to 0.77)[c]	0.65 (0.53 to 0.81)[c]	0.67 (0.54 to 0.84)[c]
High	0.48 (0.36 to 0.65)[c]	0.54 (0.40 to 0.72)[c]	0.62 (0.46 to 0.84)[c]

HR, hazard ratio.
[a]Model 1: crude model. Model 2: adjusted for age group, sex, and race. Model 3: adjusted further for education level, income, smoking status, drinking status, body mass index, hypertension, diabetes, and annual dentist visits.
[b]The lowest tertile was used as the reference group; the middle level corresponds to the second tertile; and the high level corresponds to the highest tertile.
[c]$P < 0.05$, vs. reference group.

similar among those with and without periodontal disease. Fourth, although several sociodemographic, anthropometric, and behavioral factors were adjusted for, several of which were self-reported, residual confounding may still exist.

In summary, we grouped IgG antibodies against 19 periodontal microorganisms using empirical methods and found that the orange-blue cluster, comprising *E. nodatum* and *A. naeslundii*, was inversely associated with overall cancer mortality. If further studies establish that higher antibodies against these microorganisms are protective against periodontal disease and its possible systemic effects and that this relationship is causal, then interventions to raise these antibodies may be possible.

Author Contributions

Z. Zhong, Q. Jin, contributed to data analysis and interpretation, drafted the manuscript; J. Zhang, contributed to design, data acquisition, and analysis, critically revised the manuscript; Y.M. Park, contributed to design, data

analysis, and interpretation, critically revised the manuscript; D. Shrestha, contributed to design, data acquisition, and interpretation, critically revised the manuscript; J. Bai, contributed to data interpretation, critically revised the manuscript; A.T. Merchant, contributed to conception, design, data analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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