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Periodontal Antibodies and All-Cause and Cardiovascular Disease Mortality

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Abstract

Periodontitis is positively linked to cardiovascular disease (CVD), diabetes, cancer, and increased mortality. Empirically derived clusters of IgG antibodies against 19 selected periodontal microorganisms have been associated with hyperglycemia. We further investigated associations between these serum IgG antibody clusters and all-cause and CVD mortality in a representative US population. Participants free of CVD and cancer and aged ≥40 y at baseline (N = 6,491) from the Third National Health and Nutrition Examination Survey (1988 to 1994) were followed up until December 31, 2011. Antibodies were categorized into 4 clusters: red-green, orange-red, yellow-orange, and orange-blue. Over a 23-y follow-up, 2,702 deaths occurred, including 810 CVD-related deaths. In fully adjusted Cox proportional hazard models, the red-green cluster was positively associated with all-cause mortality (tertile 3 vs. tertile 1: hazard ratio [HR] = 1.43, 95% CI = 1.08 to 1.90, P = 0.015). The yellow-orange cluster was inversely associated with all-cause mortality (tertile 3 vs. tertile 1: HR = 0.78, 95% CI = 0.63 to 0.97, P = 0.028) and CVD mortality (tertile 2 vs. tertile 1: HR = 0.57, 95% CI = 0.42 to 0.77, P = 0.005). The orange-blue cluster (composed of antibodies against *Eubacterium nodatum* and *Actinomyces naeslundii*) was inversely associated with all-cause mortality (tertile 3 vs. tertile 1: HR = 0.65, 95% CI = 0.55 to 0.78, $P < 0.0001$) and CVD mortality (tertile 3 vs. tertile 1: HR = 0.65, 95% CI = 0.47 to 0.88, P = 0.007). These antibodies could predict prognosis or be potential intervention targets to prevent systemic effects of periodontal disease if further studies establish a causal relationship.

Keywords: immunoglobulin G, periodontitis, biomarkers, cluster analysis, oral health, humoral immune response

Introduction

Periodontitis is a chronic inflammatory condition initiated by microorganisms and characterized by inflammation and destruction of supporting tooth structures, which can lead to tooth loss (Dewhirst et al. 2010; Lockhart et al. 2012). Almost half of American adults aged ≥30 y are affected by periodontitis (Eke et al. 2012). Periodontal infection is also positively linked to a series of systemic conditions, such as cardiovascular disease (CVD), diabetes mellitus, metabolic syndrome, pneumonia, adverse pregnancy outcomes, and cancer (Cullinan et al. 2009; Shrestha et al. 2015; Heikkilä et al. 2018). Infection with *Porphyromonas gingivalis* accelerated atherosclerosis in apolipoprotein E-null mice (Lalla et al. 2003), and improvement in clinical and microbial periodontal status was shown to decrease the rate of carotid artery intima-media thickness progression (Desvarieux et al. 2013).

Enhanced serum IgG antibody production induced by periodontal pathogens is a plausible protector against subsequent periodontal diseases (Papapanou et al. 2004; Rams et al. 2006). Serum IgG antibodies against periodontal microorganisms can remain elevated for up to 15 y (Papapanou et al. 2004) and may thus be used 1) to screen for periodontal infection (Kudo et al. 2012), 2) as a surrogate marker for clinical periodontal status in epidemiologic studies (Papapanou et al. 2001), or 3) for monitoring effectiveness of treatment (Kinane et al. 1999).

However, as the mouth harbors approximately 700 microorganisms, identifying relevant serum IgG antibodies to serve as surrogate markers is challenging (He et al. 2015). In a previous investigation, we grouped 4 clusters of serum IgG antibodies against 19 selected periodontal microorganisms based on the way that they grouped in vivo (Merchant et al. 2014). Specific groups of antibodies defined this way were positively or negatively associated with hyperglycemia, metabolic syndrome,

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A supplemental appendix to this article is available online.

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and cardiovascular risk factors (Merchant et al. 2014). These clusters were found to modify the associations between periodontal disease and alcohol intake/physical activity (Merchant et al. 2016; Anderson et al. 2018). The aim of this study was to further investigate the underlying associations between these serum IgG antibody clusters and all-cause and CVD mortality in a nationally representative sample of the US population. Periodontal disease has been positively associated with CVD and all-cause mortality in this cohort, but the role of specific antibodies in this relation has not been studied (Xu and Lu 2011). We hypothesized that the antibody clusters identified in previous studies (Merchant et al. 2014; Zhong et al. 2019) would be associated with all-cause and CVD mortality.

Methods

Data Source

Data from the Third National Health and Nutrition Examination Survey (NHANES III) were used. NHANES III was conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) from 1988 to 1994 among a representative sample of the noninstitutionalized civilian US population, consisting of 39,695 individuals aged 2 mo and older. Data collection was done via household interview, medical and dental examination, and laboratory tests with strict quality control procedures. Detailed information on survey methodology is described elsewhere (Centers for Disease Control and Prevention 1992; Ezzati et al. 1992; National Center for Health Statistics 1994).

Population Description

For 8,153 NHANES III participants who were ≥40 y at time of examination, their stored serum samples were tested for IgG antibodies against 19 periodontal bacteria in 2008 (CDC and National Center for Health Statistics 2008). Our study was limited to participants with complete data on IgG antibody titers and without a history of CVD or cancer, leaving 6,491 participants in the final sample. All participants were followed up for mortality until December 31, 2011.

Exposure Measures

Cluster Formation and Naming the Clusters. The exposure measures consisted of 4 mutually exclusive clusters of antibody titers. 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 with cluster analysis.

To name these clusters, we adapted the nomenclature used by Socransky and Haffajee to describe periodontal microorganism groups related to clinical periodontal disease. Microorganisms were classified into complexes with the following color scheme: organisms related to periodontal disease, red and orange complexes; to 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).

Clusters were named per the composition of microorganisms in each cluster. Cluster 1 contained antibodies against 4 microorganisms, of which 3 were from Socransky's orange complex and 1 from the red complex, and was named the orange-red cluster (*Prevotella melaninogenica, Prevotella intermedia, Prevotella nigrescens, Porphyromonas gingivalis*). The red-green cluster (*Tannerella forsythia, Treponema denticola, Aggregatibacter actinomycetemcomitans, Eikenella corrodens, Selenomonas noxia, Veillonella parvula, Campylobacter rectus*), yellow-orange cluster (*Staphylococcus intermedius, Streptococcus oralis, Streptococcus mutans, Fusobacterium nucleatum, Micromonas micros, Capnocytophaga ochracea*), and orange-blue cluster (*Eubacterium nodatum, Actinomyces naeslundii*) were similarly named.

Calculating Cluster Score. To calculate the cluster score, we first computed *z* scores of standardized log-transformed IgG titers for all 19 microorganisms. Cluster scores were then obtained by summing *z* scores of antibodies that made up each cluster for each participant. For example, the orange-red cluster contained antibody titers against *P. melaninogenica, P. intermedia, P. nigrescens*, and *P. gingivalis; z* scores of these antibody titers were summed to obtain a score for that cluster and so on (Merchant et al. 2014).

Outcome Measures

Data on total mortality and cardiovascular mortality were obtained from the NHANES III Linked Mortality File. The file contains mortality data for all eligible NHANES III participants. Participants were followed up from the date of survey participation through December 31, 2011, or the date of death, whichever occurred first; the follow-up period was up to 23 y. Underlying causes of death were recorded with validation from the death certificate. Based on a probabilistic matching algorithm reported by the National Center for Health Statistics, 96.1% of deceased and 99.4% of living participants were correctly classified (Brown et al. 2017). Details are provided elsewhere (National Center for Health Statistics and Office of Analysis and Epidemiology 2013).

Covariates

Covariates that were adjusted in this analysis to minimize the influence of confounding included age, sex, race, educational level, income, smoking status, drinking status, body mass index (BMI), diabetes, hypertension, and annual dentist visits. Age at baseline was self-reported and categorized into middle-aged and elderly (40 to 64 y and ≥65 y). Sex (male and female) and race (Black, White, and other) were categorized per self-report. As indicators of socioeconomic status, poverty income ratio (PIR) was divided into 3 groups (≤1.3, 1.3 < PIR ≤ 3.5, >3.5) and education level into 2 groups (<12 y and ≥12 y of

completed education). Smoking status (current, former, never smoker) and drinking status (drinker and nondrinker) were also based on self-report results. Former smokers were individuals who reported that they had smoked ≥100 cigarettes in their life but did not currently smoke. Nondrinkers were individuals who reported never drinking beer/lite beer, wine/wine coolers/sangria/champagne, or hard liquor (e.g., tequila, gin, vodka, scotch, rum, whiskey, and liqueurs), and other individuals were defined as drinkers. BMI was computed from weight and height measurements 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 was based on whether the participant reported being diagnosed with these conditions by a doctor. Annual visit to a dentist was a dichotomous variable. Oral health measures were obtained from the Oral Examination component of NHANES III (CDC 1992). Periodontitis was classified as being present if the individual had severe periodontitis (≥2 interproximal sites with ≥6-mm attachment loss on different teeth and ≥1 interproximal site with ≥5-mm probing depth) or moderate periodontitis (≥2 interproximal sites with ≥4-mm attachment loss on different teeth and ≥2 interproximal sites with ≥4-mm probing depth on different teeth) by Eke and colleagues' definition (2015).

Statistical Analysis

Data management and statistical analyses were conducted with SAS 9.4 (SAS Institute). The appropriate weights for the sample and complex survey design provided by NHANES III were used in all SAS survey procedures. Survival time was measured in months from the start of follow-up until death or December 31, 2011, whichever came first. The threshold for statistical significance was 0.05.

Distribution of demographic characteristics was evaluated across tertiles of clusters scores. Descriptive statistics were estimated as weighted percentage across categories. Rao-Scott tests of association between categorical variables and tertiles of cluster scores were also calculated.

Kaplan-Meier survival curves were plotted for all-cause mortality and CVD-related mortality, as stratified by tertiles of 4 clusters scores, to visually inspect the proportional hazards assumption and demonstrate the overall unadjusted association between the exposures and all-cause and CVD-related mortality.

Cox proportional hazard models were used to estimate the hazard ratio (HR) and 95% CI, with survival time to all-cause mortality and CVD-related mortality as outcomes and with tertiles of scores for orange-red, red-green, yellow-orange, and orange-blue clusters as exposures. The first model included tertiles of the 4 clusters. The second model adjusted for sociodemographic factors (sex, race, and age group). The third model further adjusted for education level, income (PIR), smoking status, drinking status, BMI, diabetes, history of hypertension, and dentist visits. Wald *F* statistics were calculated to compare the follow-up time in months from the start of follow-up to death over tertiles of each cluster score in fully adjusted models. These analyses were repeated in subgroups of sex, age group, race, and periodontal status defined by the CDC/American Academy of Periodontology classification. Prior to fitting the Cox proportional hazard models, we checked the proportional hazards assumption by evaluating if there was a linear relationship between ranks of failure time and the Schoenfeld residuals of exposures and confounders.

The article is in compliance with the STROBE statement.

Results

The cohort of 6,491 individuals was followed up to 23 y, during which 2,702 deaths occurred (31.3%), including 810 CVD-related deaths (8.3%). At baseline, approximately 78% of individuals were aged 40 to 64 y; 45% were male; and 86% were White and 10% Black.

Table 1 describes the demographic and clinical characteristics of participants across antibody cluster score tertiles. People in the top tertiles of the yellow-orange and orange-blue clusters were more likely to be younger and nonsmokers and had higher education and income levels as compared with those in the lower tertiles. Individuals in the lowest tertile of the orange-blue cluster were more likely to have moderate or severe periodontitis as compared with those in the top tertile. Individuals in the top tertiles of orange-red and red-green clusters were more likely to be Black, male, and never smokers as compared with those in the bottom tertiles.

The unadjusted Kaplan-Meier survival curves for all-cause mortality and CVD-related mortality by tertiles for each cluster are shown in Figures 1 and 2. The overall survival was about 60% (Fig. 1), and for CVD-related mortality was >80% (Fig. 2).

The proportional hazards assumptions for all exposures and covariates were valid. Table 2 shows the estimated HR and 95% CI from Cox proportional hazard models. In the fully adjusted model, the red-green cluster was positively associated with all-cause mortality (tertile 3 vs. tertile 1: HR = 1.43, 95% CI = 1.08 to 1.90, $P = 0.0148$). No significant association was observed between the red-green cluster and CVD mortality ($P = 0.4556$), although the point estimates were similar to those observed for all-cause mortality. In contrast, inverse associations were observed between the yellow-orange cluster and all-cause mortality (tertile 3 vs. tertile 1: HR = 0.78, 95% CI = 0.63 to 0.97, $P = 0.0284$; tertile 2 vs. tertile 1: HR = 0.74, 95% CI = 0.61 to 0.88, $P = 0.0013$) and CVD mortality (tertile 2 vs. tertile 1: HR = 0.57, 95% CI = 0.42 to 0.77, $P = 0.0005$). The orange-blue cluster was inversely associated with all-cause mortality (tertile 3 vs. tertile 1: HR = 0.65, 95% CI = 0.55 to 0.78, $P < 0.0001$; tertile 2 vs. tertile 1: HR = 0.75, 95% CI = 0.62 to 0.91, $P = 0.0042$) and CVD mortality (tertile 3 vs. tertile 1: HR = 0.65, 95% CI = 0.47 to 0.88, $P = 0.0066$). The orange-red cluster was not significantly associated with all-cause mortality ($P = 0.2716$) as well as CVD mortality ($P = 0.2228$).

Subgroup analyses for all-cause and CVD mortality were further conducted by age group, race, sex, and periodontitis. The results were presented with forest plots of HRs from fully

Table 1. Descriptive Characteristics (Weighted) of Study Population.

		Red-Green, %		Orange-Red, %	
	n[a]	Tertile 1	Tertile 3	Tertile 1	Tertile 3
Cluster score range	6,491	–36.09 to –30.38	2.34 to 13.21	–27.28 to –0.65	1.27 to 10.76
All-cause mortality	2,702	35.89	29.69	34.33	29.82
CVD mortality	810	9.85	7.87	8.68	8.62
Age, y					
Middle-aged, 40 to 64	4,441	77.52	78.03	77.63	78.96
Elderly, ≥65	2050	22.48	21.97	22.37	21.04
Race					
White	4,568	90.40	81.83	91.62	77.82
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	39.98	49.07
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	28.63	18.43
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
BMI, 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
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
Periodontitis status					
No disease	3,171	54.47	50.85	59.07	46.09
Moderate or severe	3,320	45.53	49.15	40.93	53.91
		Yellow-Orange, %		Orange-Blue, %	
Cluster score range	6,491	–36.09 to –30.38	2.34 to 13.21	–27.28 to –0.65	1.27 to 10.76
All-cause mortality	2,702	39.36	28.27	42.51	23.27
CVD mortality	810	11.30	7.51	11.61	5.89
Age, y					
Middle-aged, 40 to 64	4,441	74.51	79.79	73.09	83.45
Elderly, ≥65	2,050	25.49	20.21	26.91	16.55
Race					
White	4,568	89.00	81.53	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	46.16	45.83
Female	3,549	57.56	55.19	53.84	54.17

(continued)

Table 1. (continued)

		Red-Green, %		Orange-Red, %	
	n[a]	Tertile 1	Tertile 3	Tertile 1	Tertile 3
Educational level, y					
<12	2,952	30.80	27.33	31.65	21.42
≥12	3,493	69.20	72.67	68.35	78.58
Income (poverty income)					
Low	1,717	16.44	13.00	17.78	10.17
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	30.06	17.65
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
Nondrinker	3,701	50.24	50.73	53.26	46.60
BMI, 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
Present	2,251	31.33	30.95	34.06	27.82
Annual dentist visits					
No	3,806	50.56	45.45	52.04	44.12
Yes	2,476	49.44	54.55	47.96	55.88
Periodontitis status					
No disease	3,171	53.91	51.44	50.96	56.42
Moderate or severe	3,320	46.09	48.56	49.04	43.58

All percentages are weighted.
BMI, body mass index; CVD, cardiovascular disease.
[a]Values are unweighted; total numbers may be different as a result of missing data.

adjusted models for each cluster level (lowest tertile as reference; Appendix Figs. 1 and 2). Although the results seemed qualitatively similar, associations appeared stronger for Whites, females, and people without periodontitis and attenuated for those with periodontitis.

Discussion

In this study, we found that empirically-derived IgG antibody clusters against specific periodontal microorganisms were associated with all-cause and CVD mortality. After adjusting for potential confounders, higher mortality was observed for individuals with higher levels of red-green cluster scores and lower mortality for those with higher orange-blue and yellow-orange cluster scores. Higher orange-blue cluster scores were also associated with lower CVD mortality.

The orange-blue cluster had a consistent, protective association for all-cause mortality and CVD mortality. Although it is a novel finding, the cluster defined in the same way has been inversely related with hyperglycemia and elevated fasting glucose (Merchant et al. 2014; Shrestha et al. 2015), which are risk factors for cardiovascular and all-cause mortality. The orange-blue cluster consisted of antibodies against *E. nodatum* and *A. naeslundii. A. naeslundii* has been associated with good oral health in previous studies (Dewhirst et al. 2010; Desvarieux et al. 2013), with higher levels of this species being found in individuals without periodontal disease as compared with those with it (Papapanou et al. 2000). For *E. nodatum*, although higher counts of this microorganism are present in individuals with periodontal disease (Haffajee et al. 2006), higher antibody titers against *E. nodatum* have been found in individuals without periodontal disease (Papapanou et al. 2000). Similarly, the yellow-orange cluster contains *C. ochracea*, which has been linked with healthy periodontal state (Haffajee et al. 2006; Riep et al. 2009). It is plausible, therefore, that antibodies in the orange-blue and yellow-orange clusters may protect against periodontal disease, explaining the inverse association with mortality observed in our study.

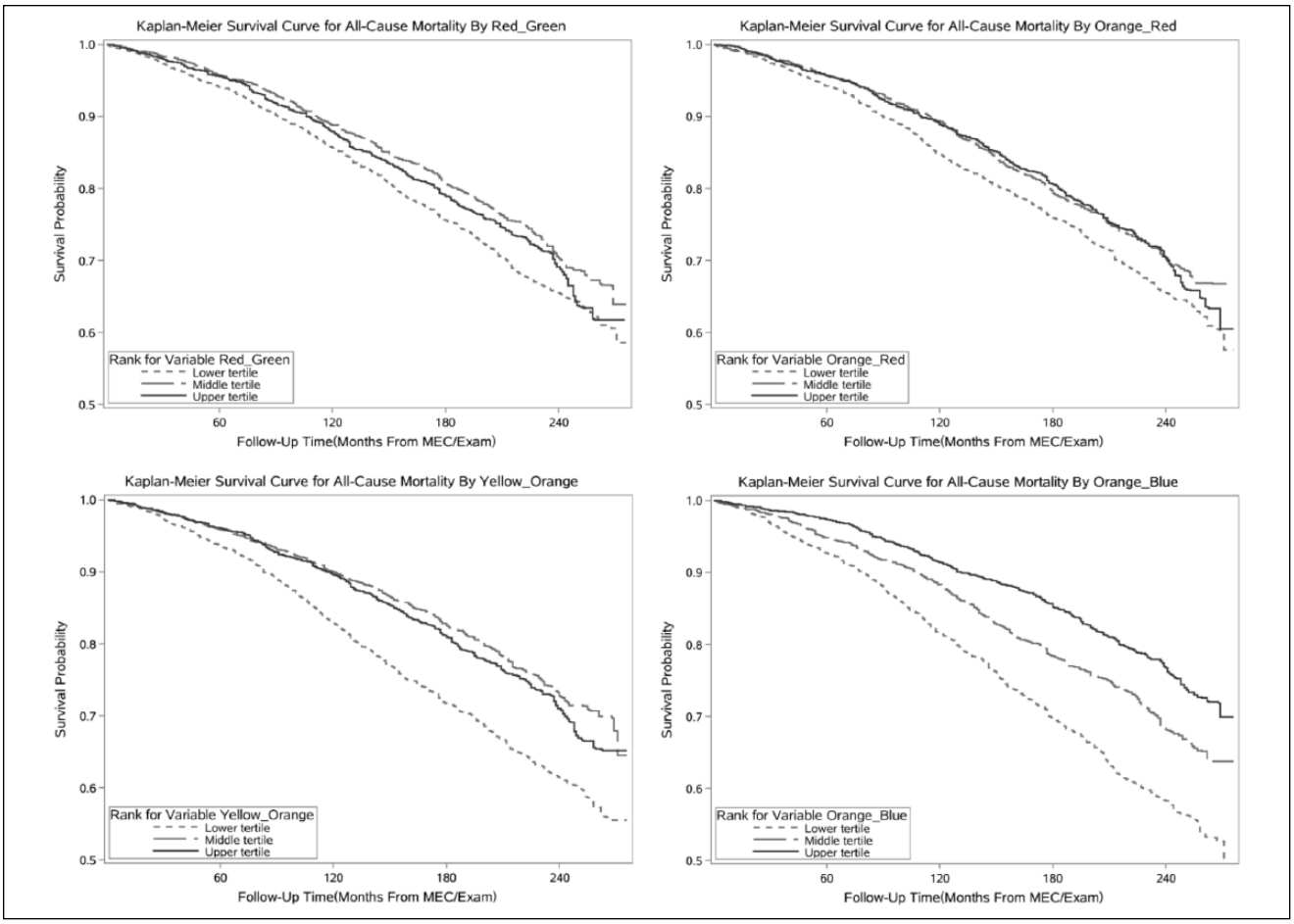

Figure 1. Kaplan-Meier survival curve for all-cause mortality by tertiles of 4 cluster scores. MEC, mobile examination center.

The red-green cluster consists of antibodies against organisms that are associated with periodontal disease, such as *T. denticola* and *T. forsythia* (Papapanou et al. 2000; Haffajee et al. 2006), and may be markers of periodontal infection, explaining the positive association with mortality. We did not observe any association between the orange-red cluster antibodies and mortality, even though *P. gingivalis* has been associated with periodontal disease and with a number of systemic conditions, including CVD, and *P. gingivalis* antibody has been associated with increased cancer mortality (Lalla et al. 2003; Ahn et al. 2012; Ishihara et al. 2014). The divergence in findings may be a result of our grouping the antibodies rather than examining them individually. The associations between subspecies of microorganisms and outcomes need to be evaluated in future studies.

Although periodontal disease is not established as a cause of CVD-related or all-cause mortality, there is evidence to support that hypothesis. Periodontal disease leads to increased levels of inflammatory markers, including CRP and TNF-alpha, and has been shown to impair insulin action (Demmer et al. 2012; Hajishengallis 2015). In addition, microorganisms associated with periodontal disease have been identified at distant sites, including the plaque in the intimal wall of blood vessels, where they are associated with inflammation, foam cell production, atherosclerosis, and plaque rupture (Kozarov et al. 2005; Kebschull et al. 2010). In randomized controlled trials, periodontal treatment has been shown to reduce oral and systemic inflammation, counts of periodontal microorganisms, hyperglycemia, and LDL-cholesterol and improve endothelial function (Merchant and Virani 2017). The association between antibodies against periodontal microorganisms and all-cause and CVD mortality needs to be interpreted within this context.

In subgroup analyses, although the general direction of the results was similar to that seen in the overall sample, the inverse associations between total mortality and orange-blue and yellow-orange clusters appeared stronger for Whites, females, and people without periodontal disease. Possible explanations for the racial differences could be attributed to the small sample of Blacks or to differences in oral flora or genetic profile (Schenkein et al. 1993). The slightly weaker associations observed between antibody clusters and mortality among males may be a result of males having a higher risk of death than females, and among those with periodontal disease,

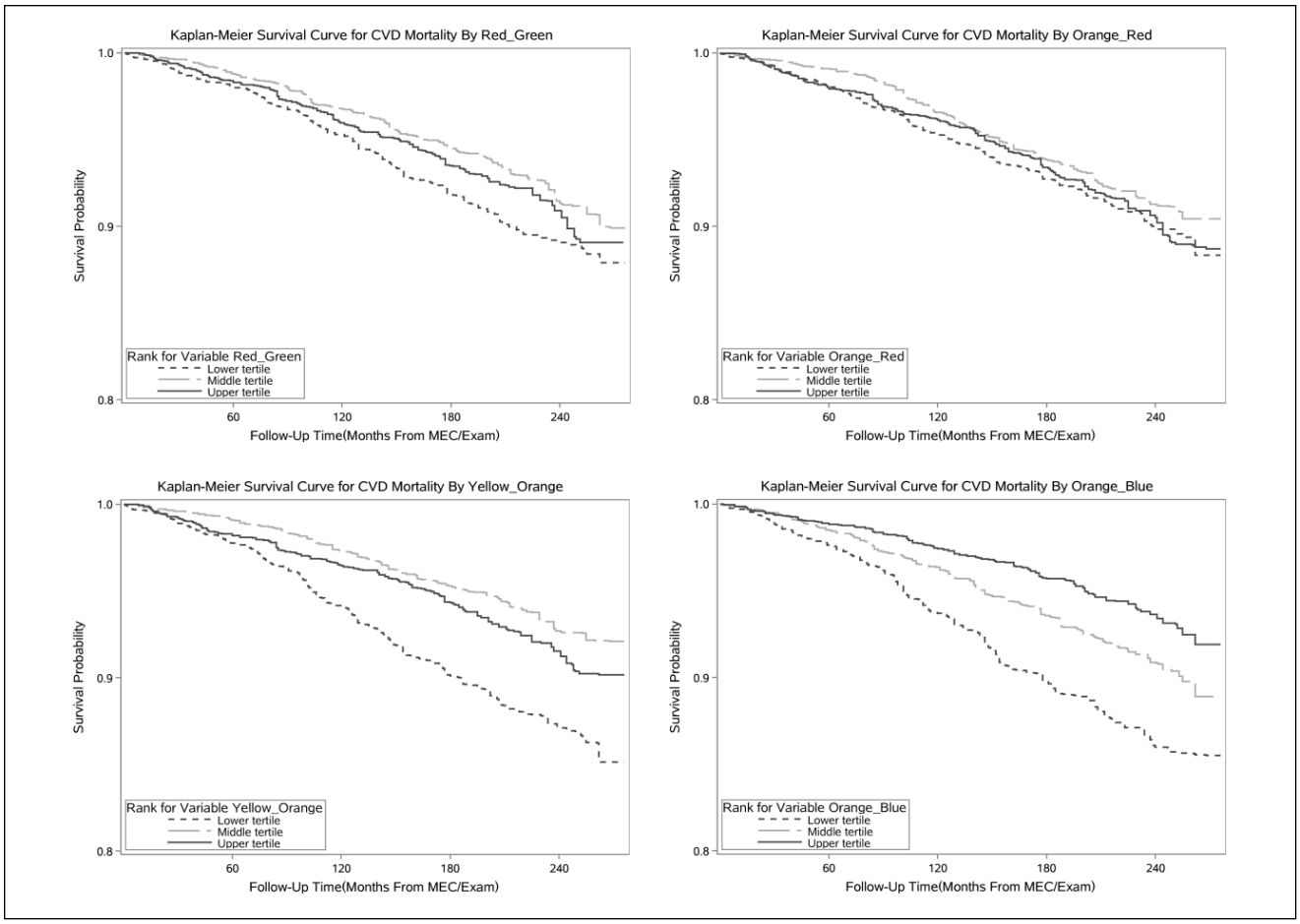

Figure 2. Kaplan-Meier survival curve for cardiovascular disease mortality by tertiles of 4 cluster scores. MEC, mobile examination center.

periodontal destruction may be on the causal pathway between the antibody clusters and mortality.

The study had several strengths. It was conducted in a representative US population; data collection was rigorous; laboratory tests and physical examinations adhered to strict standardized protocols, minimizing measurement errors. In addition, it had a prospective design with a median follow-up of 15.9 y, and outcome ascertainment was almost complete. Another advantage was the application of cluster analysis. The oral microbiome is complex, consisting of hundreds of microorganisms (He et al. 2015), which if evaluated 1 at a time would increase the chances of type 1 error because of multiple testing. Antibodies were assayed in NHANES III for 19 oral microorganisms that were well studied in relation to periodontal disease. The chances of false-positive findings were further reduced by grouping these antibodies via cluster analysis to resemble their natural grouping in vivo. The orange-blue antibody cluster was consistently associated with decreased total and cardiovascular mortality in this analysis and with hyperglycemia and fasting plasma glucose in previous reports (Merchant et al. 2014; Shrestha et al. 2015). Finally, the causal role of the orange-blue antibodies in the prevention of periodontal disease and their possible systemic effects can provide valuable information that can be explored in future studies. If the relation is determined to be causal, these antibodies could be a novel target for intervention to improve oral and systemic health. Alternatively, the orange-blue cluster antibodies may be a novel prognostic marker of CVD and mortality risk.

However, our study has some limitations. First, the current investigation included antibodies against only 19 periodontal microorganisms, which were assessed in NHANES III. It is possible that the unmeasured antibodies play a causal role or are correlated with the 19 measured antibodies. Second, although several sociodemographic, anthropometric, and behavioral factors were adjusted for, residual confounding may still exist. Third, we did not explicitly characterize the relation between antibodies against periodontal microorganisms and periodontal disease or mortality. There stands a chance that antibodies making up the clusters had opposing actions, which could have attenuated the associations. However, the association between antibodies and mortality was attenuated among people with periodontal disease, which is consistent with what would be expected if periodontal disease was on the causal pathway between antibodies and mortality. Finally, it cannot be determined whether the antibodies protect against periodontitis or indicate its presence.

Table 2. Hazard Ratios (95% CIs) of All-Cause and CVD Mortality according to 4 Cluster Scores of Antibodies against 19 Periodontal Microorganisms.

	Hazard Ratio (95% CI)		
Cluster: Level	Model 1[a]	Model 2[b]	Model 3[c]
	All-cause mortality		
Red-green			
Middle	1.18 (0.97 to 1.44)	1.09 (0.92 to 1.29)	1.11 (0.96 to 1.28)
High	1.59 (1.20 to 2.12)[d]	1.35 (1.01 to 1.81)[d]	1.43 (1.08 to 1.90)[d]
Orange-red			
Middle	0.95 (0.79 to 1.13)	0.88 (0.74 to 1.04)	0.84 (0.70 to 1.00)
High	1.00 (0.82 to 1.24)	0.87 (0.70 to 1.09)	0.89 (0.71 to 1.10)
Yellow-orange			
Middle	0.63 (0.51 to 0.77)[d]	0.76 (0.62 to 0.93)[d]	0.74 (0.61 to 0.88)[d]
High	0.63 (0.52 to 0.77)[d]	0.79 (0.63 to 1.00)	0.78 (0.63 to 0.97)[d]
Orange-blue			
Middle	0.69 (0.59 to 0.82)[d]	0.74 (0.62 to 0.89)[d]	0.75 (0.62 to 0.91)[d]
High	0.50 (0.41 to 0.61)[d]	0.58 (0.49 to 0.69)[d]	0.65 (0.55 to 0.78)[d]
	CVD-related mortality		
Red-green			
Middle	1.16 (0.90 to 1.50)	1.05 (0.83 to 1.32)	1.03 (0.79 to 1.35)
High	1.56 (0.94 to 2.59)	1.27 (0.72 to 2.26)	1.24 (0.70 to 2.21)
Orange-red			
Middle	1.12 (0.88 to 1.43)	1.03 (0.78 to 1.34)	1.05 (0.77 to 1.43)
High	1.40 (0.99 to 1.98)	1.19 (0.80 to 1.76)	1.30 (0.85 to 1.98)
Yellow-orange			
Middle	0.47 (0.34 to 0.63)[d]	0.59 (0.44 to 0.81)[d]	0.57 (0.42 to 0.77)[d]
High	0.48 (0.31 to 0.76)[d]	0.64 (0.37 to 1.10)	0.64 (0.37 to 1.08)
Orange-blue			
Middle	0.70 (0.51 to 0.95)[d]	0.75 (0.52 to 1.09)	0.77 (0.52 to 1.14)
High	0.48 (0.37 to 0.63)[d]	0.59 (0.44 to 0.79)[d]	0.65 (0.47 to 0.88)[d]

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

Conclusion

In summary, IgG antibodies against specific periodontal microorganisms that were grouped with empirical methods were associated with all-cause and CVD mortality. There was a consistent inverse association between orange-blue cluster antibodies, comprising *E. nodatum* and *A. naeslundii*, and all-cause mortality and CVD mortality. Antibodies against these microorganisms are a potential intervention target if further studies establish that this relationship is causal. These antibodies may also be markers of CVD and mortality risk.

Author Contributions

J. Qi, Z. Zihang, 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; B. Jianling, 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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