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Article

Circulating IgG antibodies to periodontal bacteria and lung cancer risk in the CLUE cohorts

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Abstract

Background: Oral health is a key indicator of overall health, well-being, and quality of life. Several studies have provided new evidence about the role of oral diseases, specifically periodontitis, in generating risk for various forms of cancers, including lung, colorectal, and pancreatic cancers.

Methods: Incident lung cancer cases (n = 192) and matched controls (n = 192) were selected from participants of the CLUE I and CLUE II cohorts. Archived serum samples collected from participants in 1974 (in CLUE I) were analyzed using immunoblotting for immunoglobulin G (IgG) antibody levels to 13 bacteria of the periodontium. Associations between antibody levels and lung cancer were estimated using conditional logistic regression.

Results: Most of the periodontal bacterial antibodies measured were inversely associated with lung cancer risk; of these, 3 were statistically significant (*Prevotella intermedia*, *Actinomyces naeslundii*, and *Veillonella parvula*). A statistically significant positive association was observed for one of the *Porphyromonas gingivalis* strains after adjusting for *P. intermedia*. The sum of the logarithm of antibodies against the 13 measured bacteria was inversely associated with risk of lung cancer when the analysis was restricted to a longer follow-up (31-44 years after blood collection, highest vs lowest quartile: odds ratio = 0.26, 95% confidence interval = 0.08 to 0.84).

Conclusions: Findings from this study highlight the complexity of using serum IgG antibodies to periodontal bacteria to identify associations between oral pathogens and risk of lung cancer. The inverse associations observed for antibodies to periodontal bacteria suggest that these may represent markers of immunity that provide some advantage in reducing the development of lung cancer.

Over the past decade, a number of studies have provided new evidence suggesting that poor oral health plays a role in generating risk for various cancers, including lung, oropharyngeal, colorectal, and pancreatic (1,2). In 2019, a meta-analysis reported that 5 out of 7 studies identified a statistically significant increase in risk of lung cancer among those with a history of periodontitis (2). The indirect mechanisms for a link between periodontitis and cancer have been attributed to the known associations between systemic inflammation and immune dysregulation, and cancer (3,4).

Serum immunoglobulin G (IgG) antibodies elicited by endotoxins of disease-causing periodontal bacteria such a *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* have been found to remain in systemic circulation up to 15 years and beyond, providing potential surrogate markers for clinical periodontitis in epidemiologic investigations (5-7). In a large prospective cohort study conducted in Europe, individuals with higher serum IgG antibodies to *P. gingivalis* had a twofold increased risk of pancreatic cancer (1). Similarly, high levels of serum IgG antibodies to *P. gingivalis* have been linked to increased risk of oropharyngeal cancers and mortality (8,9). In a recent cohort, a positive association was observed between antibodies to *Prevotella intermedia* and *Porphyromonas nigrescens* and risk of lung cancer (per interquartile range: hazard ratio = 1.15, 95% confidence interval [CI] = 1.04 to 1.26) (10).

Because levels of serum IgG antibodies to *P. gingivalis*, *T. denticola*, *T. forsythia*, and other disease-causing periodontal bacteria may reflect the severity and history of periodontitis, we sought to examine whether the presence and levels of serum IgG antibodies to these bacteria are associated with lung cancer risk 15 or more years later. Recent findings also point to changes in antibody levels with periodontal disease progression, suggesting that low antibody levels may reflect failure to mount a sustained immune response to periodontal pathogens (11). To address this

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hypothesis, we examined the association between total antibody levels to the most pathogenic periodontal bacteria and lung cancer risk 15 to 44 years later.

Methods

Study population and design

This case-control study was nested in 2 larger prospective cohort studies termed CLUE I and CLUE II. These cohorts were initiated to investigate potential serologic risk factors of cancers, heart disease, and stroke (12). Informed consent was received from each study participant. The institutional review board at the Johns Hopkins Bloomberg School of Public Health approved both the CLUE I and II studies and this study on periodontal pathogens and lung cancer risk. CLUE I (1974 to present) and CLUE II (1989 to present) were conducted in Washington County, Maryland. There was 25 620 and 32 894 study participants in each cohort, respectively (12). A total of 8394 participants overlapped between the 2 cohorts; each study recruitment was independent, and no effort was made to recontact CLUE I participants (the overlap was due to individuals voluntarily responding to both calls). Mobile office trailers were used and operated in all areas of the county to collect blood samples (15-20 mL) from every participant at the start of each cohort, and these have been stored at −70°C (12). For this analysis, we used the serum samples collected in the 1974 CLUE I study and started disease follow-up at enrollment in CLUE II for participants who took part in both CLUE I and II (to align with a separate study aim that required blood availability from 1989 and incident cancers). To be eligible, participants at the start of follow-up for CLUE II could never have had a cancer diagnosis (except nonmelanoma skin cancer).

Lung cancer ascertainment

For both CLUE I and II, incident cancer cases were ascertained by linkage to the Washington County Cancer Registry (from the start of study) and, to increase coverage for participants who may have moved out of Washington County, to the Maryland Cancer Registry (starting in 1992, when the registry began). Cancer deaths were initially identified from state vital statistics, next of kin, and obituaries. Cause of death was abstracted from the underlying cause recorded on the death certificate. We selected all lung cancer cases diagnosed after the date of CLUE II blood collection (ie, 1989) through 2018. Using incidence-density sampling, we selected 1 control per case matched on CLUE II values for age (±3 years), sex, race, smoking status, smoking intensity (number of cigarettes per day for current and former smokers), and date of blood draw (±120 days); controls selected did not have any cancer diagnosis at the time their matched case was diagnosed but could become a lung cancer case at a later time, given that the sampling was based on incidence density (13). A total of 229 incident primary lung cancer cases, all confirmed by pathology reports, were ascertained, and 229 matched controls were selected. A total of 192 lung cancer cases and 192 matched controls with results on IgG antibodies were included in the analysis (24 cases and 12 controls did not have sufficient serum remaining to measure antibodies, and 13 cases and 25 controls were removed from the analysis because they did not have a matched pair).

Selection of periodontal bacteria and assay for serum IgG antibodies to periodontal bacteria

We selected 13 bacteria based on their known associations with periodontal disease: *P. gingivalis* (ATCC 33277), *P. gingivalis* (W50), *T. denticola*, *T. forsythia*, *P. intermedia*, *Parvimonas micra*, 2 strains of *Aggregatibacter actinomycetemcomitans* (*A.a.*) (ATCC 29523 and ATCC 43718 [Y4]), *Actinomyces naeslundii*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Selenomonas noxia*, and *Veillonella parvula*.

For some of the analyses, we grouped bacteria according to the microbial color complexes described by Socransky; red complex bacteria: *P. gingivalis* 33277, *P. gingivalis* W50, *T. denticola*, and *T. forsythia*; orange complex bacteria: *P. intermedia*, *P. micra*, *C. rectus*, and *F. nucleatum*; purple complex bacteria: *V. parvula*; and blue complex bacteria: *A. naesludii* (14,15). Periodontal pathogens belonging to the red and orange complexes are considered more virulent and have been causally associated with periodontitis; purple and blue complex bacteria are associated with healthy periodontium (14,15).

We used the checkerboard immunoblotting technique (16) to assay thawed serum specimens for IgG antibodies to 13 periodontal bacteria. Two-day-old colonies of each bacterial species were harvested from blood-agar plates, suspended in phosphate buffered saline (pH 7.4), and sonicated on ice for 10 seconds with a micro ultrasonic cell disrupter (Fisherbrand, Pittsburgh, PA, USA). The optical density of each suspension was estimated using a spectrophotometer (Genesys 10S UV-VIS, Thermo Fisher Scientific) and adjusted to 1.0 at 600 nm. Bacterial suspensions were prepared fresh before use. A standard and each bacterium suspension were loaded into nitrocellulose membranes. Detection was conducted using enhanced chemiluminescence (ECL) Western blotting detection reagents and the Amersham Imager 680 workstation. Serum antibody levels for each participant were estimated using the standard curve from the purified human IgG preparations, where the standards ranged from 500 ng/mL to 7.81 ng/mL, and were analyzed using the Phoretix software. The Spearman rank correlation coefficients for repeated measurements of the bacterial antibodies were greater than 0.73 (except for *C. rectus*, *S. noxia*, and *T. denticola*, which were between 0.61 and 0.63).

To evaluate within-person variation in antibody levels over time, Spearman rank correlation coefficients and intraclass correlations were estimated using paired serum and plasma samples collected 15 years apart in 22 participants who took part in CLUE I and CLUEII. Intraclass correlations for the 13 antibodies ranged between 0.36 and 0.98, and Spearman correlations ranged between 0.45 and 0.87.

Assessment of potential confounders and other variables

Attained education, cigarette smoking status, cigarette smoking dose (number of cigarettes smoked per day), and cigar or pipe smoking status were recorded for each participant at baseline in both CLUE I and CLUE II. We classified education as less than high school or high school graduate and beyond. Participants were classified into current, former, or never cigarette smokers, and current, former, and never cigar or pipe smokers. Pack-years of cigarette smoking was obtained from the questionnaires collected during the CLUE II recruitment in 1989. Self-reported weight and height were recorded in CLUE II from which body mass index was calculated as weight in kilograms divided by height in meters squared. In CLUE II, interviewers asked women if they used postmenopausal hormone therapy. Additional data were collected in the CLUE II study in 1996, 1998, 2000, 2003, and 2007; study participants received self-administered mailed questionnaires with questions on family history, medical issues, medication, vitamin use, and exercise. On the 2003 and 2007 CLUE II questionnaires, participants were asked about periodontal

disease diagnosis. The 2003 questionnaire asked participants to report when diagnosis of periodontitis was first made and when tooth lost first occurred (1989 or before; 1990-1994; 1995 or after). The 2007 questionnaire asked participants to report when diagnosis of periodontitis was first made.

To empirically quantify pack-years of cigarette smoking among study participants, data on smoking-related DNA methylation were used to generate a smoking score. The score was derived from methylation levels measured in blood samples collected in 1989. Details on estimation of this score in the CLUE II dataset was previously published (17).

Statistical analysis

Statistical analyses were performed using STATA/SE 17.0. All hypotheses were tested using a 2-sided test, and a *P* value less than or equal to .05 was considered statistically significant. Differences in distributions of case and control characteristics that were not matched were analyzed using the χ^2 test and analysis of variance. Descriptive and summary statistics, Shapiro-Wilk normality test for distribution of antibody titers, and conditional logistic regression were performed to evaluate the risk of lung cancer associated with concentration of serum IgG antibody to each periodontal bacterium. Wilcoxon signed rank tests were conducted to test for differences in antibody concentration between lung cancer cases and controls.

Results

Table 1 shows characteristics of the incident lung cancer cases and matched controls. Of the cases, 56.1% were women, reflecting the higher participation of women in the CLUE cohorts, and the majority were White, reflecting the demographics of Washington County at the time of CLUE I and II recruitment. Cases and controls did not statistically significantly differ on body mass index, periodontitis status (33 years after baseline), or history of hormonal therapy use. As expected, cases and controls statistically significantly differed in their smoking-related DNA methylation score ($P < .01$). Among current and former smokers, cases and controls differed on the number of pack-years smoked.

Table 2 describes the quartile distribution of prediagnostic serum IgG antibody levels between lung cancer cases and controls. *A.a.* ATCC 43718 (Y4) shows the highest median antibody concentration in both cases (17.94 ng/mL) and controls (23.00 ng/mL), and *S. noxia* shows the lowest median concentration in both cases (0.64 ng/mL) and controls (0.96 ng/mL). Antibody levels to *P. intermedia*, *T. denticola*, *T. forsythia*, and *V. parvula* were statistically significantly lower in cases than controls.

We examined the association between quartiles of antibodies to each periodontal bacterium and lung cancer risk using conditional logistic regression to adjust for matching factors (Table 3). Individual bacterial antibodies were not positively associated with lung cancer overall, although several positive trends were observed among men, including a statistically significant association for *P. gingivalis* (ATCC 33277) when continuously modelled ($P = .04$). In a stratified analysis on smoking status (current vs former or never smokers), no positive association was observed for *P. gingivalis* among former or never smokers; associations were positive among current smokers, but none were statistically significant (data not shown). Inverse associations were noted for 9 of the 13 antibodies measured, with 3 (*P. intermedia*, *A. naeslundii*, and *V. parvula*) reaching statistical significance when comparing the highest with lowest quartiles and for the continuous variables. Inverse trends for these 3 antibodies were slightly stronger in men than women. When simultaneously adjusting for *P. intermedia* and *P. gingivalis* (ATCC 33277), both associations strengthened and the overall association between *P. gingivalis* (ATCC 33277) and lung cancer became statistically significant ($P = .03$, OR for 1 SD increment: 1.51, 95% CI = 1.04 to 2.21).

Finally, the sum of the log-transformed antibody levels of the oral bacteria assayed in this study was examined in relation to lung cancer risk (Table 4). When we grouped the sum of log-transformed antibody levels into quartiles, inverse associations were observed in the highest 3 quartiles compared with the bottom quartile; these associations were not statistically significant overall. The associations were similar after further adjusting for cigarette dose (Table 4) or for the DNA-derived smoking score (data not shown; numbers were smaller because not all cases and controls had DNA methylation data). The associations were similar in men and women (highest vs lowest quartile comparison, men: OR = 0.38, 95% CI = 0.10 to 1.35; women: OR = 0.48, 95% CI = 0.19 to 1.25; data not shown). Stratifying on time from blood draw to diagnosis and using 30 years as the cutpoint, the associations for total antibodies and lung cancer were strengthened and statistically significant (highest vs lowest quartile comparison: OR = 0.26, 95% CI = 0.08 to 0.84) when cases were diagnosed with a longer lag time (31-44 years). Results for total antibody levels were similar to the overall findings among former or never smokers and weaker in current smokers (Supplementary Table 1, available online). In addition, we saw similar trends with adenocarcinoma lung cancers (n = 60), which are less strongly associated with smoking; results for non-small cell carcinomas were very similar to the overall results because most lung cancers are non-small cell carcinomas (n = 143; data not shown). When stratified on median age at baseline, associations were slightly more inverse for those 42 years and older than among participants younger than 42 years (Supplementary Table 2, available online). The red complex measure was not associated with lung cancer risk, and the orange complex was inversely associated with lung cancer (data not shown).

Discussion

In this nested case-control study, we observed that many of the antibodies measured were inversely associated with lung cancer risk, with the strongest associations for *P. intermedia*. The sum of log-transformed IgG antibody levels to periodontal bacteria included in this study was also inversely associated with lung cancer risk. A suggestive positive association was observed for *P. gingivalis* (ATCC 33277) in the overall analysis; adjusting for antibodies to *P. intermedia* resulted in a statistically significant positive association for *P. gingivalis* (ATCC 33277).

A number of studies have assayed circulating IgG antibodies to periodontal bacteria using various techniques, such as checkerboard immunoblotting, dot blotting, and enzyme-linked immunosorbent assay, to examine the association between IgG antibodies to periodontal pathogens and risk of coronary artery disease (18), atherosclerosis and hypertension (19), diabetes (20), rheumatoid arthritis (21), chronic kidney disease (22), and Alzheimer disease (23). A history of periodontitis as well as the presence of circulating IgG antibodies to key periodontal pathogens have been associated with increased risk for various types of cancers, including pancreatic, oropharyngeal, esophageal, and colorectal cancers (2). In a large prospective cohort study conducted in Europe by Michaud et al. (1) to examine the association between oral bacteria and pancreatic cancer, participants with high levels of IgG antibodies against *P. gingivalis* (ATTC 53978)

Table 1. Demographic and clinical characteristics of lung cancer cases and matched controls nested in CLUE II

Characteristics at start of follow-up in 1989 (CLUE II)[a]	Lung cancer cases (n = 192)	Controls (n = 192)	P
Age, mean (SD), y	57.7 (10.1)	55.3 (10.1)	Matched
Age, mean (SD) at blood draw (1974), y	42.9 (10.1)	40.6 (10.1)	Matched
Female	56.1%	56.1%	Matched
White race	98.4%	100.0%	Matched
Cigarette smoking status			Matched
Never smoker	9.3%	9.3%	
Former smoker	37.0%	37.0%	
Current smoker	53.7%	53.7%	
No. of cigarettes smoked per day, Mean (SD)	22.7 (14.5)	22.2 (14.2)	Matched
Periodontitis status[b]			.145
Absent	71.7%	77.2%	
Present	28.3%	22.8%	
Smoking DNA methylation values, mean (SD)[c]	6.2 (50.5)	−8.1 (45.0)	.007
Pack-years smoked among current and former smokers, mean (SD)	43.7 (24. 9)	35.0 (27.6)	.002
History of HT among female participants			.35
Never	73.9%	76.0%	
Current	13.0%	17.4%	
Former	4.4%	3.3%	
Unknown	8.7%	3.3%	
BMI category			.78
Underweight	1.8%	1.8%	
Normal weight	40.2%	43.3%	
Overweight	43.3%	37.8%	
Obesity	14.7%	17.1%	

[a] Unless otherwise indicated, characteristics are from 1989 (CLUE II), which was 15 years after the collection of serum that was used to measure antibodies. For pack-years smoked, data were available for only 77 former smokers (out of 142) and 113 current smokers (out of 206). Smoking DNA methylation scores were available on 124 former smokers and 190 current smokers. Body mass index (BMI) was available on 164 cases and 164 controls. HT = hormonal therapy: estrogen, progesterone, or estrogen and progesterone (does not include oral contraceptive use).
[b] A first diagnosis of periodontitis was assessed on follow-up questionnaires in 2003 and 2007. A total 145 participants (62 controls and 83 cases) did not respond to those questionnaires (more than one-half of missing data were participants who died before 2003).
[c] Mean DNA methylation score values for cases and controls reflect predicted pack-years of smoking.

Table 2. Prediagnostic levels of serum antibodies to periodontal bacteria of lung cancer cases and matched controls in CLUE II[a]

	Lung cancer cases (n = 192)					Controls (n = 192)					
Periodontal bacterium	1%	25%	50%	75%	99%	1%	25%	50%	75%	99%	P[b]
P. gingivalis (ATCC 33277)	0.00	0.75	2.20	5.26	93.65	0.00	0.61	2.11	5.20	209.78	.63
P. gingivalis (W50)	0.47	2.29	3.71	7.11	106.90	0.68	2.29	3.86	6.70	145.95	.65
T. forsythia	0.17	1.27	2.62	4.58	21.67	0.08	1.60	2.99	5.07	35.98	.03
T. denticola	0.14	1.04	2.00	3.67	12.46	0.00	1.29	2.56	4.33	30.90	.03
P. intermedia	0.27	2.51	5.35	8.83	24.35	0.46	3.50	6.37	11.54	54.90	<.001
P. micra	0.05	4.73	11.23	20.78	155.78	1.02	5.44	10.18	20.56	155.57	.99
C. rectus	0.07	1.07	2.08	4.05	23.16	0.05	1.22	2.45	4.42	33.83	.36
F. nucleatum	0.00	0.87	1.85	3.55	26.33	0.00	1.07	2.00	4.43	22.14	.36
V. parvula	0.45	2.09	4.19	8.60	92.51	0.71	2.61	4.41	9.98	45.91	.04
A. naeslundii	0.00	0.26	0.81	2.72	48.06	0.00	0.45	1.24	3.09	24.60	.07
S. noxia	0.00	0.23	0.64	1.59	14.44	0.00	0.22	0.96	1.58	7.88	.23
A. actinomycetemcomitans (29523)	0.99	6.83	14.33	33.64	1027.69	1.47	8.67	17.52	37.55	2934.89	.51
A. actinomycetemcomitans (43718 Y4)	1.98	9.03	17.94	45.47	568.17	2.73	11.85	23.00	43.67	568.17	.17

[a] Serum from 1974, 15 years before the start of follow-up in 1989.
[b] P values from Wilcoxon signed rank test.

had 2 times the risk of developing pancreatic cancer compared with those with lower levels.

The red complex bacterium *P. gingivalis* and the orange complex bacterium *P. intermedia* are highly virulent species that have been associated with severe periodontal disease, local and systemic inflammation, and higher circulating levels of IgG antibodies. Interestingly, we observed divergent results for the antibodies to these 2 bacteria and lung cancer risk. We also noted inverse associations for antibody levels to *V. parvula* and *A. naeslundii*. In the pathogenesis of lung cancer, colonization of the lower respiratory microbiota with commensal species in the oral environment is commonly seen, and ex vivo models buttress the fact that some of these bacteria can stimulate host transcriptomic signatures linked with carcinogenesis (24). In line with our study findings, a study conducted by Zhong et al. (9) that examined serum IgG antibodies against periodontal bacteria and cancer mortality found that *A. naeslundii* was inversely associated with overall cancer mortality. In a recent prospective cohort study on lung cancer risk (10), antibodies against *P. intermedia* and *P. nigrescens* were positively associated with risk (118 lung cancer cases);

Table 3. Association between quartiles of serum IgG antibodies to periodontal bacteria and lung cancer risk 15–44 years later in CLUE II[a]

	Overall (192 cases, 192 controls)		Male (83 cases, 83 controls)		Female (109 cases, 109 controls)	
Antibodies to periodontal bacterium	**OR (95% CI)[b]**	**P[c]**	**OR (95% CI)[b]**	**P[c]**	**OR (95% CI) [b]**	**P[c]**
Red complex						
P. gingivalis 33277		.21		.04		.78
Q1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Q2	1.71 (0.92 to 3.16)		4.04 (1.40 to 11.69)		0.95 (0.42 to 2.17)	
Q3	1.47 (0.79 to 2.75)		3.26 (1.06 to 10.00)		0.95 (0.43 to 2.10)	
Q4	1.43 (0.76 to 2.86)		3.13 (0.98 to 9.34)		0.92 (0.42 to 2.04)	
P. gingivalis W50		.62		.45		.22
Q1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Q2	0.97 (0.52 to 1.83)		1.97 (0.74 to 5.20)		0.51 (0.21 to 1.27)	
Q3	0.86 (0.44 to 1.67)		1.81 (0.67 to 4.93)		0.43 (0.17 to 1.23)	
Q4	1.00 (0.54 to 1.88)		2.18 (0.82 to 5.80)		0.52 (0.22 to 1.25)	
T. forsythia		.06		.17		.22
Q1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Q2	0.60 (0.33 to 1.07)		0.43 (0.18 to 1.01)		0.80 (0.35 to 1.80)	
Q3	0.58 (0.33 to 1.00)		0.40 (0.16 to 0.99)		0.74 (0.36 to 1.48)	
Q4	0.53 (0.28 to 1.00)		0.42 (0.17 to 1.03)		0.65 (0.26 to 1.61)	
T. denticola		.14		.67		.10
Q1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Q2	1.00 (0.55 to 1.83)		0.77 (0.32 to 1.87)		1.24 (0.54 to 2.84)	
Q3	0.55 (0.29 to 1.07)		0.57 (0.21 to 1.49)		0.56 (0.23 to 1.39)	
Q4	0.57 (0.28 to 1.14)		0.56 (0.19 to 1.65)		0.58 (0.23 to 1.48)	
Orange complex						
P. intermedia		<.001		.01		.02
Q1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Q2	0.57 (0.32 to 1.02)		0.42 (0.16 to 1.13)		0.64 (0.30 to 1.35)	
Q3	0.68 (0.37 to 1.26)		0.64 (0.27 to 1.49)		0.76 (0.32 to 1.84)	
Q4	0.32 (0.16 to 0.65)		0.46 (0.17 to 1.29)		0.23 (0.08 to 0.63)	
P. micra		.23		.65		.04
Q1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Q2	0.71 (0.41 to 1.22)		1.14 (0.47 to 2.78)		0.52 (0.25 to 1.08)	
Q3	0.95 (0.54 to 1.67)		1.21 (0.54 to 2.70)		0.78 (0.34 to 1.80)	
Q4	0.92 (0.52 to 1.64)		2.40 (0.92 to 6.28)		0.51 (0.23 to 1.10)	
C. rectus		.19		.69		.15
Q1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Q2	0.94 (0.52 to 1.70)		1.25 (0.52 to 2.98)		0.66 (0.28 to 1.54)	
Q3	0.71 (0.39 to 1.28)		1.34 (0.56 to 3.21)		0.40 (0.17 to 0.94)	
Q4	0.78 (0.42 to 1.44)		1.28 (0.53 to 3.08)		0.48 (0.20 to 1.67)	
F. nucleatum		.55		.58		.18
Q1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Q2	0.68 (0.40 to 1.18)		1.40 (0.60 to 3.23)		0.37 (0.17 to 0.80)	
Q3	0.86 (0.48 to 1.54)		2.59 (1.01 to 6.61)		0.35 (0.15 to 0.81)	
Q4	0.58 (0.30 to 1.09)		1.02 (0.39 to 2.66)		0.34 (0.13 to 0.85)	
Purple complex						
V. parvula		.03		.03		.39
Q1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Q2	0.34 (0.17 to 0.66)		0.44 (0.16 to 1.23)		0.28 (0.11 to 0.72)	
Q3	0.69 (0.38 to 1.25)		0.62 (0.28 to 1.37)		0.71 (0.29 to 1.77)	
Q4	0.34 (0.17 to 0.71)		0.12 (0.03 to 0.48)		0.51 (0.20 to 1.29)	
Blue complex						
A. naeslundii		.03		.06		.25
Q1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Q2	0.44 (0.24 to 0.83)		0.37 (0.16 to 0.83)		0.37 (0.16 to 0.83)	
Q3	0.42 (0.21 to 0.82)		0.33 (0.13 to 0.80)		0.33 (0.13 to 0.80)	
Q4	0.43 (0.23 to 0.81)		0.48 (0.21 to 1.08)		0.48 (0.21 to 1.08)	
Other bacteria						
S. noxia		.54		.16		.57
Q1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Q2	1.59 (0.89 to 2.85)		1.10 (0.46 to 2.62)		2.20 (0.98 to 4.94)	
Q3	0.53 (0.26 to 1.06)		0.50 (0.18 to 1.37)		0.55 (0.21 to 1.46)	
Q4	0.92 (0.48 to 1.75)		0.68 (0.27 to 1.69)		1.19 (0.47 to 3.02)	

(continued)

Table 3. (continued)

Antibodies to periodontal bacterium	Overall (192 cases, 192 controls) OR (95% CI)[b]	P[c]	Male (83 cases, 83 controls) OR (95% CI)[b]	P[c]	Female (109 cases, 109 controls) OR (95% CI)[b]	P[c]
A. actinomycetemcomitans (29523)		.23		>.99		.14
Q1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Q2	0.88 (0.51 to 1.54)		1.19 (0.48 to 3.00)		0.80 (0.39 to 1.64)	
Q3	0.49 (0.26 to 0.91)		0.53 (0.22 to 1.83)		0.51 (0.21 to 1.26)	
Q4	0.69 (0.37 to 1.27)		1.62 (0.58 to 4.54)		0.41 (0.18 to 0.94)	
A. actinomycetemcomitans (43718 Y4)		.15		.44		.23
Q1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Q2	0.35 (0.18 to 0.69)		1.21 (0.47 to 3.13)		0.66 (0.32 to 1.33)	
Q3	0.80 (0.46 to 1.41)		0.37 (0.13 to 1.07)		0.34 (0.14 to 0.85)	
Q4	0.86 (0.46 to 1.63)		1.27 (0.41 to 3.90)		0.74 (0.34 to 1.62)	

[a] Serum from 1974, 15 years before the start of follow-up in 1989. CI = confidence interval; OR = odds ratio; antibody concentration: Q1 = first quartile; Q2 = second quartile; Q3 = third quartile and Q4 = fourth quartile.
[b] Odds ratios are from conditional logistic regression models, which took into account the matching factors (age, sex, smoking status in 1989, and number of cigarettes smoked per day).
[c] P values are for the log-transformed antibodies (for each bacteria) modeled as continuous variables in the conditional logistic regression models.

Table 4. Associations between total concentrations of antibodies to 13 oral bacteria and lung cancer risk, overall and stratified on years between blood draw and cancer diagnosis

Antibodies to oral bacteria	No. of cases/controls	OR[a] (95% CI)	Additional smoking adjustment OR[b] (95% CI)
Total antibodies	192/192	0.97 (0.93 to 1.01)	0.98 (0.94 to 1.02)
Quartiles			
1	63/48	1.00	1.0
2	44/48	0.59 (0.31 to 1.12)	0.58 (0.29 to 1.16)
3	43/48	0.58 (0.31 to 1.08)	0.53 (0.27 to 1.05)
4	42/48	0.50 (0.24 to 1.04)	0.51 (0.23 to 1.13)
P_{trend}		.08	.12

	Cases diagnosed 15-30 y after blood draw		Cases diagnosed 31-44 y after blood draw	
Antibodies to oral bacteria	No. of cases/controls	OR[b] (95% CI)	No. of cases/controls	OR[b] (95% CI)
Total antibodies	90/90	0.98 (0.92 to 1.04)	102/102	0.97 (0.92 to 1.02)
Quartiles				
1	28/24	1.00	35/24	1.0
2	22/20	0.96 (0.33 to 2.75)	22/28	0.37 (0.14 to 1.00)
3	18/26	0.63 (0.25 to 1.56)	25/22	0.46 (0.15 to 1.41)
4	22/20	0.95 (0.30 to 3.00)	20/28	0.26 (0.08 to 0.84)
P_{trend}		.77		.05

[a] Odds ratios are from conditional logistic regression models, which took into account the matching factors (age, sex, smoking status in 1989, and number of cigarettes smoked per day in 1989). CI = confidence interval; OR = odds ratio.
[b] Odds ratios were additionally adjusted for smoking status in 1974 (never, past, current <20 cigarettes per day, current ≥20 cigarettes per day; to obtain a tighter control).

the positive associations were stronger in a short follow-up time analysis (<9.9 years). The median time between blood draw and lung cancer was 17.5 years in that study, which is very different from this study, where follow-up did not start until 15 years after blood draw.

Serum IgG antibodies to periodontal bacteria have been validated by some studies as reliable diagnostic markers for periodontitis (6,25). In recent years, although several studies have looked at the association between periodontal disease and lung cancer, no study has specifically evaluated the association between serum IgG antibodies to periodontal pathogens and lung cancer risk. In a systemic review and meta-analysis conducted by Wang et al. (26) that involved 6 prospective cohort studies and 2 case-control studies, the adjusted estimates showed a positive association between periodontitis and lung cancer risk in both cohort studies (HR = 1.40, 95% CI = 1.25 to 1.58, I^2 = 8.7%) and case-control studies (OR = 1.51, 95% CI = 1.16 to 1.98, I^2 = 36.5%).

The inflammatory response associated with periodontal infections is complex and involves both innate and acquired immunity (27,28). Elevated serum IgG antibodies to periodontal bacteria are therefore potential biomarkers that may reflect the state of immunity and the capacity of the immune system to recognize early premalignant cellular clones. Hence, circulating IgG antibodies to periodontal pathogens may reflect the intersectionality of the host immune response, genetic determinants, and environmental factors.

This study has several strengths; it is prospective, and hence the findings are unlikely to be due to reverse causation due to the 15-year lag. Because clinical diagnosis of cases was confirmed by histopathological reports and incident lung cancer cases were

ascertained by cancer registry linkage, errors in the classification of outcomes were largely reduced. Moreover, because serum IgG antibodies to all periodontal bacteria considered in this study were assayed blind using checkerboard immunoblotting, a technique that has been validated (7,16) and used in numerous studies in epidemiology and periodontology, exposure misclassification bias was minimized.

This study also has some weaknesses. In both CLUE I and CLUE II, baseline assessment of confounding factors such as education, cigarette smoking status, cigarette smoking dose, weight, and height were based on self-reports. Although we adjusted for smoking status and cigarette dose in 1974 and in 1989 (through matching), we cannot rule out residual confounding by smoking; smoking is a strong risk factor for periodontal disease [light and heavy smokers have threefold and sevenfold higher risks of developing severe alveolar bone loss compared with nonsmokers, respectively (29)], and smoking has been associated with periodontal disease–associated bacterial infections [eg, a threefold increase odds in periodontal disease was noted in individuals with *A.a.* infections; although weaker, and non-statistically significant, associations were observed for *F. nucleatum* and *P. gingivalis* (30)]. In addition, smoking has been inversely associated with IgG levels (31). However, our results for the total antibodies were stronger among former or never smokers, suggesting that residual confounding is less likely to account for the inverse associations reported.

Another study limitation was that no clinical diagnosis of periodontitis was established at baseline for study participants; this would have included a comprehensive intra-oral examination, measurement of clinical attachment loss, probing depths where indicated, and dental radiographs. Participants were only asked about periodontal disease through mailed questionnaires in 2003 and 2007; by that time, many participants were deceased or did not respond to the questionnaire. If definitive diagnosis of periodontitis had been made at baseline, we could have explored the association between clinically confirmed periodontitis and serum IgG antibodies to periodontal microbiota in relation to incident lung cancer cases. Lastly, because levels of antibodies vary over time, using a one-time measurement may not accurately capture the association between periodontal disease and cancer risk. However, results from our validation study comparing measurements from blood collected 15 years apart suggest that antibody levels track over time.

In this case-control study that was nested in the CLUE I and II cohorts, we identified inverse associations between circulating IgG antibodies against *P. intermedia*, *A. naeslundii*, and *V. parvula* and lung cancer risk and a positive association for one of the *P. gingivalis* strains (ATCC 33277). Additionally, a decrease in lung cancer risk was observed between the sum of log-transformed IgG antibodies to periodontal microbiota and risk of cancer 31-44 years after the first blood draw (in CLUE I). More studies with repeated measures of antibodies to periodontal disease bacteria, periodontal disease assessment, updated information on smoking, and very long follow-up periods will be needed to understand the complex relationship between smoking, periodontal disease, the immune response, and lung cancer risk.

Data availability

The data cannot be deposited into a controlled access database due to a State of Maryland law that established the Maryland Cancer Registry (where the lung cancer data were obtained). Researchers who are interested in conducting analyses using the CLUE cohort data will need to apply for approval and should contact Dr Elizabeth Platz (eplatz1@jhu.edu).

Author contributions

Nana Ampomah, BDS, MPH (Formal analysis; Writing – original draft); Flavia Teles, DDS (Data curation; Methodology; Writing – review & editing); Lynn Martin, BA (Data curation; Methodology; Writing – review & editing); Jiayun Lu, PhD (Data curation; Writing – review & editing); Devin Koestler, PhD (Methodology; Writing – review & editing); Karl Kelsey, MD (Conceptualization; Funding acquisition; Writing – review & editing); James Beck, DDS (Conceptualization; Methodology; Writing – review & editing); Elizabeth Platz, ScD (Conceptualization; Funding acquisition; Project administration; Resources; Writing – review & editing); Dominique S Michaud, ScD (Conceptualization; Data curation; Formal analysis; Funding acquisition; Project administration; Supervision; Writing – review & editing).

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Conflicts of interest

Dr Kelsey is a founder and scientific advisor for Cellintec, which had no role in this research.

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