

Periodontal Disease and Breast Cancer: Prospective Cohort Study of Postmenopausal Women

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Abstract

Background: Periodontal disease has been consistently associated with chronic disease; there are no large studies of breast cancer, although oral-associated microbes are present in breast tumors.

Methods: In the Women's Health Initiative Observational Study, a prospective cohort of postmenopausal women, 73,737 women without previous breast cancer were followed. Incident, primary, invasive breast tumors were verified by physician adjudication. Periodontal disease was by self-report. HRs and 95% confidence intervals (CI) were estimated by Cox proportional hazards, adjusted for breast cancer risk factors. Because the oral microbiome of those with periodontal disease differs with smoking status, we examined associations stratified by smoking.

Results: 2,124 incident, invasive breast cancer cases were identified after mean follow-up of 6.7 years. Periodontal disease, reported by 26.1% of women, was associated with

increased breast cancer risk (HR 1.14; 95% CI, 1.03–1.26), particularly among former smokers who quit within 20 years (HR 1.36; 95% CI, 1.05–1.77). Among current smokers, the trend was similar (HR 1.32; 95% CI, 0.83–2.11); there were few cases ($n = 74$) and the CI included the null. The population attributable fraction was 12.06% (95% CI, 1.12–21.79) and 10.90% (95% CI, 10.31–28.94) for periodontal disease among former smokers quitting within 20 years and current smokers, respectively.

Conclusion: Periodontal disease, a common chronic inflammatory disorder, was associated with increased risk of postmenopausal breast cancer, particularly among former smokers who quit in the past 20 years.

Impact: Understanding a possible role of the oral microbiome in breast carcinogenesis could impact prevention. *Cancer Epidemiol Biomarkers Prev*; 25(1); 43–50. ©2015 AACR.

Introduction

Periodontal disease is a highly prevalent, chronic condition characterized by altered oral microbiota and a proinflammatory environment (1). It has been found to be associated with increased risk of systemic chronic diseases, including heart disease (2, 3), stroke (4), and diabetes (5). While there has been less study of the association of periodontal disease with cancer, there is evidence that those with the disease are at increased risk of oral, esophageal, head and neck, pancreatic, and lung cancers (6–10). There has been limited study of periodontal disease and breast cancer. In three prospective studies, there was a nonstatistically significant increased risk of breast cancer among those with

periodontitis; all three were small in size and limited in power (11–13). We examined the association between self-reported periodontal disease history and breast cancer risk in the Women's Health Initiative Observational Study (WHI OS), a large prospective cohort of postmenopausal women in the United States.

Materials and Methods

Study population

The WHI OS has been described in detail elsewhere (14, 15). Briefly, it is a prospective cohort study of 93,676 postmenopausal women, volunteers aged 50 to 79 years, enrolled at 40 centers throughout the United States between 1994 and 1998. The study was approved by the Institutional Review Boards of each of the centers and written informed consent was obtained from all participants before participation in the study.

Ascertainment of study exposures and outcomes

Study participants completed extensive self-administered questionnaires, physical examinations, and blood collection (16, 17). Participants have been followed annually to ascertain additional exposure information and to determine changes in health status. History of periodontal disease diagnosis was determined on a questionnaire completed at year five of follow-up. Included in the analyses reported here were study participants who completed the questionnaire regarding periodontal disease and who had no history of breast cancer at the time of the periodontal disease

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report ($n = 73,737$). Excluded were women who did not complete the questionnaire ($n = 11,262$), did not complete the dental questions ($n = 1,208$), had been diagnosed with breast cancer prior to year five ($n = 6,621$), or were lost to follow-up ($n = 848$). For these analyses, participants were followed through September 30, 2010.

Diagnosis of breast cancer among study participants was determined by self-report on questionnaires collected annually (18) and were verified by review of medical records by trained physician adjudicators using the International Classification of Diseases for Oncology, second edition (ICD-O-2) and classified using guidelines from the Surveillance, Epidemiology, and End Results program (19). Data collected included tumor type, stage, nodal status, tumor size, estrogen receptor (ER), progesterone receptor (PR), and HER2 status. There were 2,124 diagnosed cases of confirmed, incident, primary, invasive breast cancer after the year five assessment of periodontal disease among women with no history of breast cancer.

Assessment of history of periodontal disease diagnosis was by self-report using a validated questionnaire (20). Participants responded to the query: "Has a dentist or dental hygienist ever told you that you had periodontal or gum disease?" In addition, they provided information regarding frequency of dental care and loss of all permanent teeth.

Data regarding other potential confounding factors [age, education, race/ethnicity (to predefined categories), age at menarche, menopause, and first birth, parity, family history of breast cancer, alcohol consumption, physical activity, smoking history, and second-hand smoke exposure] were obtained from self-administered questionnaires; information regarding postmenopausal hormone use and use of aspirin and/or nonsteroidal anti-inflammatory drugs (NSAID) were obtained from standardized, interviewer-administered questionnaires. Body height and weight were measured using a clinical balance beam scale and stadiometer, and body mass index (BMI) was calculated as $\text{weight (kg)}/[\text{height (m)}]^2$. Smoking status was updated each year. Participants were classified as never, current, or former smokers at the time of the fifth year questionnaire. Among those who had ever smoked cigarettes, pack-years of smoking were calculated as packs smoked per day multiplied by number of years smoked. Second-hand smoke exposure was assessed in childhood and at home and work during adulthood. Categories of quantitative variables (those not posed as categorical variables on the WHI questionnaires) were developed based in part on combining groups with similar biology (e.g., similar biology for age at menarche) and in part on developing categories of equal size to the extent possible. These categorical variables were included in adjusted models. For individuals with missing age at menopause or age at menarche, age was imputed using the median for the cohort. For other variables, those with missing values for an adjusting variable were not included in the full model analysis.

Statistical analysis

Characteristics of study participants with and without periodontal disease were compared using χ^2 tests for categorical variables and Student *t* tests for continuous variables. Follow-up time was computed as the time between completion of the year five questionnaire and the first occurring study endpoint: breast cancer diagnosis, death from any cause, loss to follow-up, or end of follow-up. Hazard ratios (HR) and 95% confidence intervals (CI) were computed with Cox proportional hazards regression,

adjusting for breast cancer risk factors (age, education, race/ethnicity, body mass index, age at menarche, age at menopause, parity, age at first birth, postmenopausal hormone use, alcohol consumption, physical activity, and use of NSAIDs). We also examined models further adjusting for family history of breast cancer, personal history of diabetes, stroke, and myocardial infarction, frequency of dental visits, second-hand smoke exposure, and edentulism.

Because smoking is associated with periodontal disease (21), we examined control for the potential impact of smoking on the association between periodontal disease and breast cancer risk using several approaches. We examined smoking modeled as smoking status (current/former/never), as years smoked and packs per day entered separately, as pack-years of smoking (alone). Because there might be differences in the oral microbiota with periodontal disease for smokers compared to nonsmokers (22, 23), we examined models stratified on smoking status.

In addition, we examined models excluding all women with any history of cancer, and excluding those diagnosed with breast cancer in the year after the ascertainment of periodontal disease status. We examined multiplicative interaction of the association of periodontal disease and breast cancer by age, race, family history of breast cancer, BMI, physical activity, use of hormone therapy, alcohol, and NSAIDs by calculation of the *P* for the multiplicative interaction term. We examined models stratified by ER status, edentulism, frequency of dental visits, and mammography, examining differences in the strata by calculation of the *P* for the multiplicative interaction term.

Finally, we determined population attributable fraction of incident breast cancer among those with a history of periodontal disease, computed as $P_c(1 - 1/\text{HR}_{\text{adj}})$ where P_c is the prevalence of periodontal disease among the breast cancer cases and HR_{adj} is the multivariable-adjusted HR for the association of periodontal disease and breast cancer (24, 25). All statistical analyses were performed in SAS 9.3 (SAS Institute).

Results

Characteristics of study participants are shown in Tables 1 and 2. Because of the large sample, several comparisons were significantly different although differences were small. Mean follow-up from the time of periodontal disease assessment was 6.7 years. Mean follow-up time was slightly longer (0.2 years) among those with periodontal disease compared with those without. Approximately 26% of all participants reported having been told that they had periodontal disease, 21% of never smokers, 30% of former smokers, and 38% of current smokers (data not shown). Those without periodontal disease were on average 0.9 years older than those with the disease. There were statistically significant differences between those with periodontal disease and those without for age at menopause, education, race/ethnicity, age at menarche, age at first birth, parity, mammography, hormone therapy, alcohol consumption, routine dental checks, edentulism, and smoking. Among those with invasive breast cancer, periodontal disease status was not associated with tumor characteristics (Table 3).

The association of periodontal disease and invasive breast cancer is shown in Table 4. There was a significant increase in risk of invasive breast cancer among those reporting a history of periodontal disease; the adjusted HR was 1.14 (95% CI, 1.03–1.26); it was somewhat weaker with adjustment for smoking

Table 1. Participant characteristics by periodontal disease, WHI OS

	Total (n = 73,737)	Periodontal disease		P ^a
		Yes (n = 19,262)	No (n = 54,475)	
	Mean ± SD	Mean ± SD	Mean ± SD	
Follow-up (years) ^b	6.7 ± 2.7	6.8 ± 2.6	6.6 ± 2.7	<0.001
Age (year 5)	68.7 ± 7.2	68.1 ± 7.0	69.0 ± 7.3	<0.001
BMI (kg/m ²)	27.3 ± 5.8	27.3 ± 5.8	27.3 ± 5.8	0.29
Physical activity (MET hours/week)	13.3 ± 13.8	13.4 ± 13.6	13.3 ± 13.9	0.34
	N (%)	N (%)	N (%)	
Education				
≤High school diploma	14,770 (20.2)	2,982 (15.6)	11,788 (21.8)	<0.001
College or some college	35,342 (48.3)	9,088 (47.5)	26,254 (48.6)	
Post-graduate	23,058 (31.5)	7,048 (36.9)	16,010 (29.6)	
Race				
American Indian/Alaskan Native	304 (0.4)	75 (0.4)	229 (0.4)	<0.001
Asian/Pacific Islander	2,096 (2.9)	459 (2.4)	1,637 (3.0)	
Black	4,895 (6.7)	1,483 (7.7)	3,412 (6.3)	
White, not of Hispanic origin	63,062 (85.7)	16,447 (85.6)	46,615 (85.8)	
Unknown	816 (1.1)	216 (1.1)	600 (1.1)	
Any aspirin or nonsteroidal anti-inflammatory use, year 3				
<1 year current use	44,551 (66.5)	11,750 (67.0)	32,801 (66.3)	0.06
1 or more years current use	22,476 (33.5)	5,777 (33.0)	16,699 (33.7)	
Alcohol consumption past 3 months, year 3				
None	21,829 (31.0)	4,910 (26.8)	16,919 (32.5)	<0.001
<1/2 drink/day	30,932 (44.0)	8,121 (44.3)	22,811 (43.8)	
≥1/2 drink/day	17,613 (25.0)	5,306 (28.9)	12,307 (23.7)	
Routine dental check-ups				
2 or more times per year	50,183	14,939 (77.6)	35,244 (64.7)	<0.001
Once per year	11,090	1,802 (9.4)	9,288 (17.1)	
Less than once per year	2,107	468 (2.4)	1,639 (3.0)	
Never in past three years	4,580	867 (4.5)	3,713 (6.8)	
Whenever needed	5,777	1,186 (6.2)	4,591 (8.4)	
Edentulous				
Yes	5,059 (6.9)	1,089 (5.7)	3,970 (7.3)	<0.001
No	68,678 (93.1)	18,173 (94.3)	50,505 (92.7)	
Smoking status, year 5				
Never smoked	37,376 (51.3)	7,941 (41.7)	29,435 (54.7)	<0.001
Former smoker	32,400 (44.5)	9,900 (52.0)	22,500 (41.8)	
Current smoker	3,087 (4.2)	1,180 (6.2)	1,907 (3.5)	
Former smokers, years since quit, year 5				
Quit ≥20 years ago	23,524 (73.1)	6,622 (67.3)	16,902 (75.7)	<0.001
Quit <20 years ago	8,638 (26.9)	3,219 (32.7)	5,419 (24.3)	
Smokers, pack-years, year 5				
≤5	11,474 (33.7)	2,834 (26.6)	8,640 (36.9)	<0.001
>5–24	11,242 (33.0)	3,431 (32.2)	7,811 (33.4)	
>24	11,355 (33.3)	4,405 (41.3)	6,950 (29.7)	

NOTE: Number of missing data for BMI, $n = 122$; physical activity, $n = 228$; education, $n = 567$; race, $n = 188$; any aspirin or NSAID use, year 3, $n = 6,710$; alcohol consumption, $n = 3,363$; smoking status, $n = 874$; years since quit, $n = 238$; pack-years 1,724.

^aP value for Student t test for continuous variables and for χ^2 test for categorical variables.

^bFollow-up from year 5 to breast cancer, end of follow-up period or death in years.

status and pack-years (HR 1.11; 95% CI, 1.00–1.23). Adjustment for smoking with separate variables for years smoked and packs per day did not measurably change the findings (data not shown). Results were similar after adjusting for history of other cancer, diabetes, stroke or myocardial infarction, family history of breast cancer, second-hand smoke exposure, frequency of dental visits, or edentulism (data not shown).

In analyses stratified by smoking status (Table 5), there was a small, nonsignificant increase in risk of breast cancer risk associated with periodontal disease among never smokers (HR 1.06; 95% CI, 0.91–1.24). For former smokers who had quit more than 20 years previous, there was also a nonsignificant increase in risk (HR 1.08; 95% CI, 0.91–1.27). Among former smokers who had quit within the past 20 years, there was a 36% increase in risk

(HR 1.36; 95% CI, 1.05–1.77). Among current smokers, the magnitude of the association was similar to that for former smokers who had quit within the last 20 years; however, the number of cases was small ($n = 74$) and the CI was wider and included the null (HR 1.32; 95% CI, 0.83–2.11). The test for multiplicative interaction was not significant ($P = 0.40$). In models that also included adjustment for pack-years of smoking, results were similar although the confidence intervals were wider and included the null (data not shown).

There was no evidence of multiplicative interaction of the association of periodontal disease with breast cancer and age, race/ethnicity, family history of breast cancer, BMI, physical activity, use of hormone therapy, alcohol consumption, or use of NSAIDs. There were no differences in the associations in strata

Table 2. Participant reproductive characteristics by periodontal disease, WHI OS

	Total (n=73,737)	Periodontal disease		P ^a
		Yes (n=19,262)	No (n=54,475)	
Age at menopause	Mean ± SD 48.3 ± 6.2	Mean ± SD 48.4 ± 6.1	Mean ± SD 48.3 ± 6.2	0.01
	N (%)	N (%)	N (%)	
Age at menarche				
9-11	16,145 (21.9)	4,529 (23.5)	11,616 (21.3)	<0.001
12-13	41,119 (55.8)	10,694 (55.5)	30,425 (55.9)	
≥14	16,473 (22.3)	4,039 (21.0)	12,434 (22.8)	
Age at first birth				
Never pregnant or never had term	9,196 (12.6)	2,664 (13.9)	6,532 (12.1)	<0.001
<20	7,983 (10.9)	2,009 (10.5)	5,974 (11.0)	
20-29	50,519 (69.0)	12,918 (67.5)	37,601 (69.5)	
≥30	5,547 (7.6)	1,539 (8.0)	4,008 (7.4)	
Parity				
Never pregnant or never had term	9,196 (12.6)	2,664 (13.9)	6,532 (12.1)	<0.001
1-2	26,011 (35.5)	7,090 (37.1)	18,921 (35.0)	
3-4	28,544 (39.0)	7,237 (37.8)	21,307 (39.4)	
≥5	9,494 (13.0)	2,139 (11.2)	7,355 (13.6)	
Family history of breast cancer				
Yes	13,173 (18.8)	3,427 (18.8)	9,746 (18.9)	0.84
No	56,717 (81.2)	14,803 (81.2)	41,914 (81.1)	
Mammograms in last 5 years				
1-3	22,544 (32.9)	5,777 (32.0)	16,767 (33.2)	0.005
>3	46,065 (67.1)	12,265 (68.0)	33,800 (66.8)	
Hormone use at year 5				
Never used hormones	20,318 (28.4)	5,228 (27.9)	15,090 (28.6)	<0.001
Former E-alone user	7,270 (10.2)	1,684 (9.0)	5,586 (10.6)	
Current E-alone user	15,508 (21.7)	3,893 (20.8)	11,615 (22.0)	
Former E+P user	12,935 (18.1)	3,521 (18.8)	9,414 (17.8)	
Current E+P user	15,526 (21.7)	4,394 (23.5)	11,132 (21.1)	

NOTE: Number of missing data for age at first birth and parity, $n = 492$; family history of breast cancer, $n = 3,847$; mammogram in last 5 years, $n = 5,128$; hormone use at year 5, $n = 2,180$.

^aP value for Student t test for continuous variables and for χ^2 test for categorical variables.

defined by ER status, edentulism, frequency of dental visits, or of mammography (data not shown).

The population attributable fraction, the portion of breast cancer that would be eliminated if periodontal disease were removed and all other factors remained the same was 2.89% (95% CI, -0.06-5.75) for the total population, 1.30% (95% CI, -2.29-4.76) for never smokers, 4.74% (95% CI, 0.11-9.16) for all former smokers, 2.25% (95% CI, -2.83-7.08) for former smokers who quit more than 20 years previous, 12.06% (95% CI, 1.12-21.79) for former smokers who quit less than 20 years previous, and 11.47% (95% CI, -10.31-28.94) for current smokers (Table 5).

Discussion

In the WHI OS, a large, well-characterized prospective cohort of postmenopausal women, reported history of diagnosis of periodontal disease was associated with primary, invasive breast cancer. Among former smokers who had quit smoking in the previous 20 years, there was a 36% increase in risk. The association was similar among current smokers but the number of women in this category was smaller; the CI was wider and included the null. These findings are consistent with a role of chronic inflammation in breast cancer risk and point to a possible role of the oral microbiome in breast cancer etiology and prevention.

Study strengths and limitations should be taken into account in interpretation of these findings. Strengths of the study include the

prospective design, the completeness of follow-up, the large population size and the well-characterized cohort such that we were able to examine both confounding and interaction by known risk factors. Adjudication of all incident breast cancer cases ensured that there is little misclassification in outcome measures. Furthermore, because this is a generally health conscious cohort that receives frequent medical care, data regarding other breast cancer risk factors, while again by self-report, is generally well measured.

This study was limited to postmenopausal women; findings can only be generalized with confidence to that group. Furthermore, the volunteer participants in the WHI observational cohort tended to have some difference in their health behaviors. Prevalence of several periodontal disease risk factors including smoking, diabetes, and obesity were lower in the study than in the general population. While these differences may limit study generalizability, there is no reason to think that the biologic processes would be different. Another potential limitation is the possibility of confounding in estimates of risk. While we examined potential confounding by all known risk factors for breast cancer and periodontal disease, there may be additional unknown confounders. There could also be residual confounding by smoking, by education, or socioeconomic status. Pack-years measure of smoking history is closely correlated with severity of periodontal disease; adjusting for it may be over control and result in an underestimate of the association between periodontal disease and breast cancer risk. On the other hand, residual confounding by

Table 3. Characteristics of invasive breast tumors (stage, lymph node involvement, tumor size, ER, PR, and HER2 status), by periodontal disease status, WHI OS

	Total (n = 2,124)	Periodontal disease		P ^a
		Yes (n = 616)	No (n = 1,508)	
Stage				
Localized	1,590	454 (75.3)	1,136 (76.7)	0.51
Regional or distant	495	149 (24.7)	346 (23.3)	
Missing	39	13	26	
Nodal status				
Negative	1,445	418 (75.2)	1,027 (76.5)	0.53
Positive	453	138 (24.8)	315 (23.5)	
Missing	226	60	166	
Tumor size				
<2 cm	1,431	414 (71.6)	1,017 (71.8)	0.54
2–4.9 cm	498	141 (24.4)	357 (25.2)	
≥5 cm	66	23 (4.0)	43 (3.0)	
Missing	129	38	91	
ER/PR status				
ER+PR+	1,391	405 (70.9)	986 (69.7)	0.67
ER+PR–	290	87 (15.2)	203 (14.3)	
ER–PR+	17	4 (0.7)	13 (0.9)	
ER–PR–	288	75 (13.1)	213 (15.1)	
Missing	138	45	93	
ER status				
Positive	1,704	496 (86.0)	1,208 (84.1)	0.28
Negative	310	81 (14.0)	229 (15.9)	
Missing	110	39	71	
PR status				
Positive	1,408	409 (71.4)	999 (70.6)	0.71
Negative	581	164 (28.6)	417 (29.4)	
Missing	135	43	92	
HER2 status				
Positive	265	74 (13.8)	191 (14.6)	0.64
Negative	1,579	463 (86.2)	1,116 (85.4)	
Missing	280	79	201	

^aP value, χ^2 test.

smoking might explain some of the observed association. Smoking is strongly associated with periodontal disease; the association with breast cancer is weaker (26). With regard to education, it might also affect results if less educated women received less dental care and were not aware of their periodontal disease status. Even if true, such an association would not likely impact results greatly given that there is not a lot of variability in the cohort for these factors; the cohort is highly educated and largely receives regular dental care. Only 4% of participants had less than high school education and more than 80% had a routine dental checkup at least annually. Another issue is the determination of periodontal disease status by self-report. There is likely misclassification of exposure to periodontal disease. In a subsample of the cohort, comparisons were made between periodontal disease

Table 4. Association of periodontal disease with risk of invasive breast cancer, WHI OS

	Total N	Cases N	HR (95% CI)
Age adjusted	73,737	2,124	1.14 (1.04–1.26)
Model 1 ^a	63,800	1,898	1.14 (1.03–1.26)
Model 2 ^b	61,693	1,828	1.11 (1.00–1.23)

^aModel 1: Adjusted for age, education, race/ethnicity, BMI, age at menarche, age at menopause, parity, age at first birth, hormone use, alcohol consumption, physical activity, and NSAIDs.^bModel 2: Adjusted for variables in Model 1; additionally adjusted for smoking status and pack-years.

assessed by self-reported questionnaire and by clinical dental evaluation; sensitivity, specificity, positive, and negative predictive value of self report compared with clinically determined severe periodontal disease were 56%, 79%, 33%, and 91%, respectively, and 76%, 77%, 22%, and 97% compared with tooth loss to periodontitis (20). Misclassification of the measure of periodontal disease would likely bias results toward the null; it may be that we underestimated the strength of the association. We did not have data regarding the severity of the periodontal disease or the date when it was diagnosed, details which would have improved our ability to examine the associations.

While there is accumulating evidence that periodontal disease is associated with increased risk of cancer, particularly oral, esophageal, head and neck, pancreatic, and lung cancers as well as possible increases in prostate and hematologic cancers (6–10), there have been, to our knowledge, just three prospective studies of the association with breast cancer. Findings from all three are consistent with our findings (11–13). In follow-up of the National Health and Nutrition Examination Survey (NHANES) I Follow-up Study, clinical measurement of periodontitis was associated with a 32% increase in breast cancer risk. However, the study included only 19 breast cancer cases and the increase was not statistically significant (11). In a cohort of 838 women in Sweden, there was a statistically significant increase in breast cancer among those with periodontal disease assessed by a clinical exam; again the number of breast cancer cases was small, only 24 (13). Finally, in a prospective study of approximately 15,000 twin pairs, there was a nonsignificant increase in breast cancer risk of 12%. The latter study included 531 cases. In that study, the measure of periodontal disease was of tooth mobility, a measure with good specificity but poor sensitivity (12).

There are several potential mechanisms that could explain the observed association of periodontal disease with breast cancer. It could be that periodontal pathogens directly impact carcinogenesis. Bacteria from the oral cavity enter the blood stream following

Table 5. Association of periodontal disease with risk of invasive breast cancer, by smoking status, WHI OS

	Total N	Cases N	Case prevalence periodontal disease, %	HR ^a (95% CI)	PAF ^a (95% CI)
Smoking status					
Never smoker	32,593	907	22.9	1.06 (0.91–1.24)	1.30 (–2.29–4.76)
Former smoker	28,091	902	34.4	1.16 (1.01–1.33)	4.74 (0.11–9.16)
Quit ≥ 20 years	20,515	659	30.3	1.08 (0.91–1.27)	2.25 (–2.83–7.08)
Quit < 20 years	7,387	237	45.6	1.36 (1.05–1.77)	12.06 (1.12–21.79)
Current smoker	2,467	74	47.3	1.32 (0.83–2.11)	11.47 (–10.31–28.94)

^aHR and population attributable fractions (PAF) adjusted for age, education, race/ethnicity, BMI, age at menarche, age at menopause, parity, age at first birth, hormone use, alcohol consumption, physical activity, and NSAIDs.

activities including tooth brushing, flossing and chewing, particularly among those with periodontal disease (27). While these circulating oral bacteria are rapidly cleared, there is considerable cumulative exposure to tissues (28). It is known that milk ducts are not sterile, that breast ductal tissues are exposed to bacteria and viruses during lactation and that human milk contains a complex and variable array of microbes (29–31). Furthermore, there is evidence from small studies of the presence of bacteria in breast tissues (32–34) including in breast tumors (34). The origins of microbes in breast tissues and tumors are not known but the oral cavity and gut might contribute (30). Some of the bacteria species identified in breast tissues (33) are also found in the mouth although it is not known if there are the same strains. There is some evidence (35–37), although not consistent (38, 39), that there is an increase in breast cancer risk associated with antibiotic use. Particular antibiotics might or might not alter the oral microbiome.

Another potential mechanism is inflammation resulting from the periodontal disease impacting systemic processes including breast carcinogenesis (40). Periodontal disease is associated with chronic systemic inflammation including increased blood C-reactive protein (41, 42), cytokines and chemokines (43), with a potential impact on carcinogenesis (44). Bacterial metabolites produced in the mouth including nitrosamines and acetaldehyde could have a systemic impact on carcinogenesis (45). It could also be that there are common risk factors including smoking, physical activity, or diet as well as etiologic factors such as inflammation, oxidative stress, or shared genetic factors that contribute to host susceptibility to both breast cancer and periodontal disease (27, 46–48). The cytokine receptor activator of NF- κ B (RANK) and its ligand (RANKL) may be important in breast carcinogenesis and metastasis (49–52). Blood and salivary RANKL are increased in periodontal disease, especially among smokers (53, 54). We found that breast cancer risk associated with periodontal disease was limited to smokers, particularly former smokers who had quit in the previous 20 years. Smoking is a major risk factor for periodontal disease (21); the bacterial microbiota for periodontal patients differs for smokers and nonsmokers (22, 23). Smokers' microbiomes have less diversity, higher prevalence of organisms associated with periodontal pathogenesis, and lower prevalence of those associated with health (22, 55). There is evidence of lower humoral immune response in both current and former smokers compared with never smokers (56). Our finding of increased breast cancer risk associated with periodontal disease among former smokers who had quit in the past 20 years could be an indication that previous exposure to smoking was significant in the carcinogenic process or that the smoking resulted in a change slow to be reversed. We examined models both with and without adjusting for pack years of smoking. While point estimates were similar, the confidence intervals were wider and included the null for the latter, more adjusted model. Periodontal disease is common, particularly among older adults. In this cohort, 26% of all participants reported having been told by a dental professional that they have periodontal disease and 31% of current and former smokers reported periodontal disease. If there is a relationship between periodontal disease and breast cancer, based on our findings of attributable risk, approximately 11% of cases among current smokers and 12% of cases among former smokers would result from periodontal disease, and thus could

potentially be prevented through improved control of periodontal disease in older women.

We found increased risk of invasive breast cancer among postmenopausal women who had been told that they had periodontal disease, particularly former smokers who had quit in the previous 20 years. Replication of these findings in other populations will allow us to better understand this association between periodontal disease and breast cancer, with the potential to provide new insights and new strategies for prevention of breast cancer. These findings have potential important public health relevance as the subgroup of older U.S. women continues to grow, with increased incidence of both periodontal disease and of breast cancer. Future research should include a species and even strain specific examination of the oral microbiome, particularly for those with periodontal disease, and former and current smokers in relation to the microbiome in normal breast tissues and in breast tumors. Data regarding changes in breast tissues from animal models with treatment of periodontal disease would also be important to understand the observations reported here.

Disclosure of Potential Conflicts of Interest

R.J. Genco reports receiving a commercial research grant from Sunstar; has received speakers bureau honoraria from Sunstar, Colgate Palmolive, Johnson & Johnson, Wrigley, Cigna Insurance Co., and Proctor and Gamble; and is a consultant/advisory board member for Sunstar. These organizations might have an interest in the submitted work but did not contribute to the work. He is a member of the Scientific Advisory Panel of the American Academy of Periodontology, a nonfinancial interest which may be relevant to the submitted work. No potential conflicts of interest were disclosed by the other authors.

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Development of methodology: J.L. Freudenheim
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R.J. Genco, J. Wactawski-Wende
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.L. Freudenheim, R.J. Genco, M.J. LaMonte, K.M. Hovey, X. Mai, C.A. Andrews, J. Wactawski-Wende
Writing, review, and/or revision of the manuscript: J.L. Freudenheim, R.J. Genco, M.J. LaMonte, A.E. Millen, K.M. Hovey, X. Mai, N. Nwizu, C.A. Andrews, J. Wactawski-Wende
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.L. Freudenheim, J. Wactawski-Wende
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For a list of all the investigators who have contributed to WHI science, please visit: <https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf>.

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