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# The Buffalo OsteoPerio Studies: Summary of our findings and the unique contributions of Robert J. Genco, DDS, PhD

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## Abstract

**Purpose—**Robert ("Bob") Genco was a member of the research team that established the Buffalo Osteoporosis and Periodontal Disease (OsteoPerio) study. Here we detail the scientific discoveries emanating from this enduring collaboration.

**Study cohorts—**OsteoPerio is ancillary to the Women's Health Initiative Observational Study (WHI-OS). WHI-OS is a longitudinal study of 93,000 postmenopausal women aged 50–79 enrolled at 40 U.S. centers (enrolled 1993–1998). OsteoPerio enrolled 1342 women 3 years later (1997–2001) from the Buffalo WHI-OS participants to study the association of osteoporosis and periodontal disease. OsteoPerio has 5-year (N=1026) and 17-year (N=518) follow-up examinations.

**Participants—**In addition to information on health status from the WHI-OS, OsteoPerio further included comprehensive oral examinations assessing probing pocket depth, clinical attachment loss, gingival bleeding, alveolar crestal height, and DMFT. Systemic bone density (measured by DXA), blood, saliva and plaque also were collected at all three visits.

**Summary—**Findings from these studies are summarized here.

### Keywords

Periodontal diseases; Tooth loss; Osteoporosis; Women; Microbiome; Aging; Robert J. Genco



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## INTRODUCTION

In 1993, the University at Buffalo (UB) was selected as a vanguard center for the NIH funded “Women’s Health Initiative” (WHI) that included three overlapping randomized clinical trials and an observational study assessing the major causes of morbidity and mortality in postmenopausal women (1). JWW was a lead investigator intimately involved in establishing and implementing the WHI protocol at Buffalo and nationally. Shortly thereafter, we were approached by Dr. Robert J. (“Bob”) Genco, DDS PhD inquiring about a potential collaboration. Our first meeting followed, and so began the start of a 25 year collaboration.

Bob was long-time chair of the Department of Oral Biology at UB. In his usual gracious manner, he sent congratulations on being awarded WHI and followed with, “Was there a dental component to WHI?” There was not. “Could we find a way to collaborate?” and “When can we meet?” The first meeting included WHI investigators Maurizio Trevisan (PI) and Jean Wactawski-Wende (Co-I). Bob asked about the objectives for this then planned 12-year study (WHI has now been ongoing over 25 years). He listened intently. At the end of that meeting, we focused on two main areas of exploration – the role of periodontal disease in heart disease (MI Perio Study) with Maurizio Trevisan, and the association of osteoporosis and periodontal disease (the OsteoPerio study) with Jean Wactawski-Wende. Bob’s vision and energy to pursue these two projects proved to be inspirational throughout their enduring collaborations.

Although this meeting started with three individuals, the projects ultimately included dozens of scientific investigators; trainees from oral biology, epidemiology, biostatistics, bioinformatics, and medicine; and collaborating scientists from around the U.S. and the world.

### Funding to Support OsteoPerio

UB provided funding for preliminary data collection on 70 women who completed a comprehensive oral exam, assessed bone density by dual x-ray absorptiometry (DXA), and collected extensive risk factor information regarding bone and oral health. In Buffalo, we enrolled 2249 women into the WHI-OS and later recruited these women into the OsteoPerio study. OsteoPerio was efficient, as all data collected from WHI-OS was available, including annual assessment of health outcomes and risk factor information (NIH/NHLBI N01WH32122, 1993–2020). Funding for OsteoPerio enrollment and baseline examination came from the Department of Defense. Aims were to determine the association between periodontal disease, systemic osteoporosis and oral bone loss (Wactawski-Wende (PI), DoD #OS950077, 1997–2001). A reexamination investigated the 5-year predictors of oral and systemic bone loss and periodontal disease progression (Wactawski-Wende (PI), NIDCR R01 DE13505, 2002–2007). Several mechanistic grants were funded. These included a study of the relationship between periodontal disease and metabolic syndrome (measured glucose, insulin, C-reactive protein, and lipids) (LaMonte (PI), NIDCR R03 DE022654–2, 2012–2015); a study of the relationship between plasma Vitamin D levels and periodontal disease (Millen (PI), NIDCR R21 DE020918–02, 2010–2013); and a study to explore the correlation between serum and salivary biomarkers of inflammation and

bone metabolism and their associations with periodontal disease (Wactawski-Wende (PI), NIDCR RC1 DE020404–02, 2009–2012). Our current R01 focuses on the subgingival plaque microbiome and periodontal disease at three time points over 17 years (Wactawski-Wende (PI), NIDCR, R01 DE024523, 2014–2020 NCE). To our knowledge, this is the largest prospective epidemiological study of the oral microbiome and periodontal disease in older women. We have also partnered with colleagues to explore novel bioinformatics approaches for use of microbiome data (Sun PI, NIAID, R01 AI125982, 2016–2019).

## Study Cohorts and Participant Recruitment

The WHI-OS and the Buffalo OsteoPerio study are summarized here. UB was selected as a WHI vanguard center in 1993. From 1993 to 1998, 161,808 women enrolled at 40 U.S. centers into three overlapping randomized clinical trials (N= 68,000) or a prospective observational study (N=93,000). (1) In Buffalo, 2249 postmenopausal women were enrolled into the WHI-OS. Participants completed physical measures, a medication inventory, questionnaires on medical history and personal habits and provided blood samples. Three years post-baseline (1996–2001), similar assessments were conducted. Annually, WHI women complete questionnaires assessing major health outcomes (CVD, stroke, cancer, fracture, hospitalizations, diabetes, hypertension, among others) and update information on selected risk factors. In WHI-OS year-5 (1998–2003), questions on periodontal disease and oral health were included on periodontal disease/gum disease, edentulism, personal oral hygiene habits, and frequency of dental visits. We have used these WHI-wide data to explore the role of periodontal disease on risk of developing chronic diseases. OsteoPerio recruited participants from Buffalo WHI-OS. This included 1342 participants at baseline (1997–2001; mean age 63 years), 1026 participants 5-years later (2002–2005) and 518 women 17-years post baseline (2014–2018). (2) See Figure 1.

## OsteoPerio Data Collection

After initial telephone screening, eligible and interested participants were scheduled for clinic visits. Prior to the visit, participants were sent a consent to review, visit instructions, and study questionnaires to complete. At each visit, participants provided informed consent; completed blood pressure, pulse, and anthropometric measurements; had saliva and fasting blood collection; and completed oral examinations and bone density assessments.

**Oral Examination and sample collection—**Oral examinations included a whole-mouth assessment by calibrated dental examiners. Subgingival plaque samples were taken from 12 index teeth (or their substitutes) using a paper point technique (3). Samples were frozen for later use. Participants also provided 5 mL of whole saliva at each visit. Oral examinations assessed the head and neck, oral mucous membranes, recorded restorative appliances, and decay/caries, missing and filled teeth (DMFT). Gingival bleeding, calculus and plaque presence was recorded for each tooth. Pocket depth was measured using a constant force electronic periodontal probe (The Florida Probe System, Gainsville, FL, USA). Clinical attachment loss (CAL) was determined as the distance from the CEJ to the base of the pocket. Eleven intraoral radiographs were taken using a medical unit adapted for this purpose using a cepholostat to control head geometry. Radiographs were digitized and ACH level determined at each tooth (4). Change in ACH used the side-by-side

method described by Hausmann (5). PPD, CAL and ACH measures are available for each tooth/site, worst sites within an individual and as whole mouth mean values. Periodontal disease presence and severity were defined using either the OsteoPerio for alveolar crestal height (ACH) (6), or the Centers for Disease Control and Prevention/American Academy of Periodontology (CDC/AAP) using CAL. (7) Fasting blood samples were drawn; centrifuged in the laboratory; portioned to serum, plasma and buffy coat, and frozen and housed in a biospecimen bank.

**Demographic and Physical Assessments—**Demographic information and personal characteristics were assessed via questionnaire. Information included demographic factors, medical and oral health history, dental hygiene, smoking, dietary intake, medication use and dietary supplements. As part of the WHI study, participants were asked questions about a wide range of medical, psychological and behavioral factors, health conditions and diseases. Anthropometric measures (height, weight, waist circumference) and blood pressure were completed according to standardized protocols. Bone density (spine, hip, wrist, total body) and body composition (bone, fat and lean mass) was assessed using DXA.

**Subgingival microbiome assessment—**The composition and diversity of the subgingival plaque microbiome was assessed by 16S ribosomal DNA (rDNA) sequencing with the Illumina high-throughput MiSeq platform in plaque samples from three time points (2). Quality control was systematically evaluated throughout the sample preparation and sequencing procedures to ensure quality for use in longitudinal epidemiologic studies. In baseline and year-5 samples, targeted immunofluorescence methods were conducted to assess the presence of 8 oral bacteria at the time of sample collection. (3, 8, 9)

## OsteoPerio findings

***Osteoporosis, systemic bone mineral density, and periodontal disease:*** At the time OsteoPerio was conceived, few studies had focused on the relationship between oral and systemic bone in older women. The association between systemic bone mineral density (BMD) and periodontal disease was the initial focus of our work. Findings from a pilot study in 70 Caucasian postmenopausal women, aged 51 to 78 years, provided initial support for our work (10, 11). Significant correlations were observed for mean ACH level and BMD of several regions of the hip (trochanter, $r = {}^{-}0.27$; Ward's triangle, $r = {}^{-}0.26$; and total femur, $r = {}^{-}0.25$; all $P<0.05$). Mean CAL appeared to be similarly associated with BMD, however the associations did not reach statistical significance.

We examined baseline systemic bone density and ACH in the OsteoPerio study in 1,341 postmenopausal women aged 53 to 85. DXA BMD measured were categorized by severity of the worst site T-score measured (normal >−1.00; low −1.00 to −2.00; moderate −2.01 to −2.49; osteoporotic <−2.5). Compared with women with normal T-scores, women with osteoporosis were nearly twice as likely to have worse ACH (OR=1.90; 95% CI: 1.19, 3.05). The relationship appeared to be greater in women over age 70 (OR=3.57; 95% CI: 1.42, 8.97) compared to women age ≤70 (OR=1.63; 95% CI: 0.94, 2.83). (6) We further explored the association of ACH and BMD according to presence of pathogenic bacteria from immunofluorescence (3). In 1,256 participants with all variables available,

both forearm BMD (Beta = ⁻0.931, p=0.038) and presence of Tannerella forsythia (Beta = 0.125, p=0.015) were associated with worse ACH after adjustment and simultaneous control of each other. In women ≤70, both lower BMD and presence of a periodontal pathogen were independent factors influencing periodontal disease.

The associations of systemic bone density and periodontal disease measures were generally stronger using ACH, as opposed to soft tissue measures. We assessed whether CAL was associated with systemic BMD in OsteoPerio. Forearm BMD was significantly associated with CAL, however only in women without calculus present (beta = ⁻1.308; P=0.001). (12)

**Vitamin D and Periodontal disease:** Vitamin D has both anti-inflammatory and antimicrobial properties that may favorably influence bone and periodontal health. We examined plasma 25(OH)D concentrations and clinical periodontal measures in 920 OsteoPerio participants. No association was observed between 25(OH)D concentration and ACH (OR = 0.96, 95% CI: 0.68, 1.35). However, women with adequate compared with deficient/inadequate 25(OH)D levels had 33% lower odds (95% CI: 5%, 53%) of periodontal disease using the CDC/AAP definition and 42% lower odds (95% CI: 21%, 58%) of having ≥50% of gingival sites that bled on probing.(13) Five-year change in periodontal measures was not associated with 25(OH)D concentration (14) or incident tooth loss. (15) There was also no association between presence of 5 subgingival pathogens and 25(OH)D concentration. (16) Despite a sound biological framework, findings in OsteoPerio have not supported role for vitamin D in periodontal disease.

**Tooth loss and periodontal disease:** We determined tooth loss was common in postmenopausal women, severity of periodontal disease was associated with loss, and certain risk factors independently increased risk of tooth loss in postmenopausal women. (17) After 5.1 years of follow-up in 1021 women, 293 women lost a total of 323 teeth (28.7% loss of at least one tooth). Each 1 mm increment (worsening) baseline ACH, CAL and PD was associated with 1.22 (95% CI 1.11, 1.35), 1.13 (95% CI 1.05, 1.23) and 1.26 (95% CI 1.13, 1.41) greater odds of losing at least one tooth. We also evaluated a tooth loss prediction model that included community-level health indicators but no clinical periodontal measures. Diabetes history (OR = 2.45, 95% CI: 1.26, 4.77), self-reported gum disease history (OR = 1.97, 95% CI: 1.43, 2.70), ever smoking (OR = 1.42, 95% CI: 1.06, 1.89), number of teeth missing at baseline (OR = 1.05 per tooth, 95% CI: 1.02, 1.08), and BMI (OR = 1.15 per 5 $kg/m^2$ increase, 95% CI: 1.01, 1.33) were each independently associated with increased risk of tooth loss. Clinical measurements of periodontal disease and questionnaire-based models provided similar risk estimates for incident tooth loss.

We also assessed the role of smoking on tooth loss. (18) In 1106 participants with information on smoking and reason for loss of each missing tooth, we found heavy smokers (≥ 26 pack-years) were more likely to experience tooth loss compared with never smokers (OR = 1.82; 95% CI: 1.10, 3.00). Smoking status, packs smoked per day, years of smoking, pack-years and years since quitting smoking were significantly associated with tooth loss due to periodontal disease. A strong positive association was evident for tooth loss due to periodontal disease when comparing heavy smokers with never smokers (OR = 6.83, 95%

CI: 3.40, 13.72). No significant associations were found for smoking and tooth loss due to caries.

**Cancer and periodontal disease:** The association between periodontal disease and cancer has been of interest by our team and others. We have examined relationships in both the WHI-OS and OsteoPerio. WHI-OS is large, has extensive follow-up and thousands of incident cancer cases, however it is limited by self-reported periodontal disease. OsteoPerio is more limited in sample size, follow-up time, and number of incident cancer cases, but is well-characterized using clinical measures of periodontal disease. In WHI-OS we explored history of periodontal disease and lung cancer incidence. (19) Prevalence of periodontal disease was reported in 26% of the WHI-OS participants and was found to be positively associated with lung cancer risk (HR = 1.24, 95% CI: 1.07–1.45). When restricted to never-smokers, periodontal disease was not associated with lung cancer (HR 1.02, 95 % CI 0.68–1.53). However, a positive additive interaction between periodontal disease and pack-years of smoking ($p = 0.02$) was observed, suggesting that in moderate-to-heavy smokers, presence of periodontal disease resulted in lung cancer risk that was greater than that observed for either exposure alone.

Breast cancer incidence was assessed in the WHI-OS women. (20) A total of 2,124 incident, invasive breast cancer cases occurred after 6.7 years. Periodontal disease 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 of WHI enrollment (HR = 1.36; 95% CI: 1.05, 1.77). WHI-OS included few current smokers who developed breast cancer (74 cases), however, the association was similar (HR 1.32; 95% CI, 0.83, 2.11). Total and site-specific cancer was assessed in 65,869 WHI-OS participants reporting no prior cancer. After 8.32 years follow-up, 7,149 incident invasive cancers were identified. Periodontal disease history was associated with risk of total cancer (HR = 1.14; 95% CI: 1.08, 1.20); findings were similar in analyses limited to 34,097 never-smokers (HR = 1.12; 95% CI: 1.04, 1.22). Significant associations were observed for breast (HR = 1.13; 95% CI: 1.03, 1.23), lung (HR = 1.31; 95% CI: 1.14, 1.51), esophagus (HR = 3.28; 95% CI: 1.64, 6.53), gallbladder (HR = 1.73; 95% CI: 1.01, 2.95), and melanoma skin (HR = 1.23; 95% CI: 1.02, 1.48) cancers. Stomach cancer had a borderline risk (HR = 1.58; 95% CI: 0.94, 2.67). (21)

We explored total cancer risk in OsteoPerio. After 12.2 years of follow-up, no statistically significant associations were found for ACH-defined categories and total cancer (mild/moderate vs. none: HR = 1.33, 95% CI: 0.91, 1.94; severe vs. none: HR = 1.20, 95% CI: 0.77, 1.86), however results were consistent in magnitude with those reported in WHI-OS. A positive association between whole-mouth mean and worst-site ACH and lung cancer (per 1 mm increment) (adjusted HR 1.81, 95% CI 1.30, 2.54; adjusted HR 1.34, 95% CI 1.08, 1.66, respectively). However, case numbers were small, and confounding by smoking could not be ruled out. (22)

Five periodontal pathogens measured by immunofluorescence were also evaluated in relation to cancer incidence. (23) Neither the presence of individual pathogens nor the presence of any of Socransky's red-complex pathogens was associated with total or site-specific cancer. (24) Borderline associations were seen for the presence of orange-complex

pathogens (F. nucleatum, P. intermedia, and C. rectus) with total cancer risk (HR = 1.35, 95% CI: 1.00, 1.84), and lung cancer risk (HR = 3.02, 95% CI: 0.98, 9.29).

**Metabolic syndrome, hypertension, CVD and periodontal disease:** A longstanding infection hypothesis of atherosclerosis (25) has been a basis for investigating an association between periodontal and cardiovascular disease. (26) In the WHI-OS we assessed the relationship between periodontal disease and CVD, stroke and mortality in over 57,000 women. A total of 3589 incident CVD events and 3816 total deaths occurred during a mean follow-up of 6.7 years. Periodontitis history was not associated with CVD events, but was associated with higher total mortality (HR=1.12, 95% CI: 1.05, 1.21). Edentulism was associated with higher age- and smoking-adjusted risks of CVD (HR=1.42, 95% CI: 1.27, 1.59) and total mortality (HR=1.47, 95% CI: 1.32, 1.63). Further adjustment eliminated the association with CVD, but mortality remained significantly increased (HR=1.17, 95% CI: 1.02, 1.33). (27)

Similarly, in 36,692 WHI-OS women without baseline hypertension, we found edentulism to be significantly associated with incident hypertension (adjusted HR=1.21, 95% CI: 1.11, 1.30). This association was stronger among women <60 years compared to ≥60 years (P interaction = 0.04), and among those whose baseline systolic blood pressure (SBP) was <120 mm compared to ≥120 mmHg (P interaction = 0.004). (28) In the OsteoPerio study we assessed the cross-sectional association of clinically measured periodontal disease severity with prevalent hypertension and measured SBP among 1341 postmenopausal women. CAL and number of teeth missing were positively associated with SBP among those not taking antihypertensive medications ($P < 0.05$). ACH and gingival bleeding on probing were associated with higher SBP in crude but not adjusted models. Periodontal disease severity categories were not associated with SBP. Number of teeth missing however was significantly associated with prevalent hypertension (adjusted OR = 1.14, per 5 teeth; P = 0.04). ACH, CAL, PPD, gingival bleeding, and severity of periodontitis were not significantly associated with prevalent hypertension in adjusted models. (29)

We have also characterized the association of metabolic syndrome and its components with periodontal disease in 657 OsteoPerio women. Metabolic syndrome was present in 25.6% of these women. In adjusted models, metabolic syndrome was significantly associated with supragingival plaque (OR = 1.47; 95% CI: 1.00, 2.16; P = 0.049), but not with ACH, CAL, PD or gingival bleeding. (30) The multivariable association between metabolic syndrome and plaque was strengthened (OR = 2.04, 95% CI: 1.12, 3.74) in women whose serum C-reactive protein concentration was ≥3.0 mg/dL, which is indicative of chronic systemic inflammation.

**Other work emanating from our collaboration with Bob Genco—**Additional work that was accomplished as part of our long collaboration included studies of reproducibility of probing measures (31), accuracy of self-reported periodontal disease in WHI-OS (32), and characterization of the 5-year changes in periodontal disease in OsteoPerio (33). Bob Genco was instrumental in the design and interpretation of a novel and innovative multiplex study assessing cytokines in serum and saliva (34) at a time when the promise of salivary diagnostics was one of his keen pursuits. (35) Bob also was a key contributor

on bioinformatics papers on approaches to assess complex sequencing and genomic data emanating from our oral microbiome work. (36, 37)

**Oral microbiome in postmenopausal women:** In the OsteoPerio early work, using an immunofluorescence technique, we found a strong positive association between specific oral bacteria and severe oral bone loss. Infection with *P. gingivalis* (OR = 2.34; 95% CI: 1.66 to 3.29), *T. forsythia* (OR = 1.76; 95% CI: 1.34 to 2.30), *P. intermedia* (OR = 1.40; 95% CI: 1.07 to 1.83) and *C. rectus* (OR = 1.85; 95% CI: 1.33 to 2.57) were associated with oral bone loss (ACH). Overweight (BMI 25.0–29.9 kg/m$^2$) women with T. forsythia infection were more likely to have oral bone loss (OR = 3.37; 95% CI: 2.08, 5.46) than those with normal weight (BMI 18.5–24.9; OR = 1.27; 95% CI: 0.82, 1.98) or who were obese (BMI ≥30; OR = 1.26, 95% CI: 0.72, 2.20). (3)

As next generation sequencing became accessible and cost feasible, and with the advent of the human microbiome project, Bob encouraged us to utilize available plaque and saliva samples to assess the microbiome. Bob was enthusiastic to characterize the oral microbiome using state-of-the-science methods in an understudied population (older women). He was convinced that many new previously uncultured/annotated microflora would be identified. We conducted a third OsteoPerio visit 17-years post-baseline to provide additional follow-up time to enhance our precision in studying periodontal progression. We initially conducted a pilot study to determine the feasibility of a larger study. Baseline subgingival plaque samples from 15 women from OsteoPerio with no periodontitis and from 15 women with severe periodontitis (determined by probing measures) were assessed. The 16S rRNA gene (V1 to V3 hypervariable region) was sequenced on the 454 FLX platform. The PICRUSt technique was used to provide information on what the potential functional characteristics of microbiota might be in healthy, compared with diseased, periodontium. There were clear differences in the plaque microbiomes associated with health as compared to periodontal disease. Of the 464 species identified, 22.8% had elevated abundance in disease and 6.3% had elevated abundance in health. Among the 12 most prevalent organisms in periodontitis, one-half have previously been recognized as periodontal pathogens. The microbiomes associated with periodontitis contained genes that could code for activities including microbial mobility, synthesis of endotoxin, and proteolytic degradation. The healthy microbiome included genes that could code for sustaining microbial life, including encoding for transporters, glycolysis, gluconeogenesis, the Krebs cycle, and protein kinases. (38)

NIDCR funding allowed us to implement the OsteoPerio Microbiome Study, including a 17-year post baseline visit. (2) Nearly 2600 subgingival plaque samples have now been characterized from plaque samples available at the three OsteoPerio time points using the 16S metagenomic sequencing technique. Initial results using early data from 446 of the baseline participant samples (39) determined the plaque microbiome in four groups stratified on BP status: normal BP (systolic < 120 and diastolic < 80; N = 179), elevated BP/Stage I hypertension (systolic 120–139 or diastolic 80–90; N = 106), Stage II hypertension (systolic > 140 or diastolic > 90; N = 42). A fourth group consisted of anyone taking hypertension medications, regardless of BP (N = 119). Sixty-five bacterial species demonstrated significant differences in relative abundance in women with elevated

BP or using hypertension medication as compared to those with normal BP. After correction for multiple testing, two species, Prevotella (oral species 317) and Streptococcus oralis remained significantly associated. They were lower in abundance among women taking anti-hypertension medications compared to those with normal BP (corrected $P < 0.05$).

Two of the primary findings of the OsteoPerio Microbiome study have recently been published. Lamonte and others (40) aimed to determine the extent to which the composition and diversity of the oral microbiome varies with age in 1219 postmenopausal women. Of the 267 species identified, *Veillonella dispar* was the most abundant bacteria (mean 8.9%); whereas *Streptococcus oralis, Veillonella dispar and Veillonella parvula* were most prevalent (100%, all) as being present at any amount. Twelve species differed across age groups after Bonferroni correction: 5 (42%) were higher in women ages 50–59 compared to ≥70 (corrected $P<0.05$), and 7 (48%) were higher in women 70 years and older.

In 2019, two weeks before his death, Bob submitted a landmark paper on the subgingival microbiome and it relationship to periodontal disease severity in postmenopausal women. (41) The composition and diversity of the subgingival microflora and their oligotypes were assessed using the 16S rRNA gene in 1,206 women (aged 53 to 81). Prevalence of none/mild, moderate, and severe periodontal disease was 25.1%, 58.3%, and 16.6%, respectively. Alpha diversity of the microbiome differed significantly across the 3 periodontal disease categories. β-Diversity differed between no/mild and severe periodontal disease, although considerable overlap was noted. Of the 267 bacterial species identified, 56 (20.9%) differed significantly in abundance according to periodontal disease status. Of the taxa differing in abundance according to periodontal disease status, 53% had multiple oligotypes appearing to differ between none/mild and severe periodontal disease.

## FUTURE PLANS

The OsteoPerio study with its rich available data, including the subgingival plaque microbiome data in a large sample of participants at three time points will be used to answer a variety of research questions focused on temporal changes and their determinants. Little is currently known about the relationship between the subgingival plaque microbiome and health outcomes beyond periodontitis in older women; data from the OsteoPerio study will begin to fill this gap. Prior to Bob’s passing, we were determining definitions of periodontal change over 17-years of follow-up. We also were planning to explore the metabolome in these women.

Dr. Robert J. Genco was a remarkable colleague; he always exuded such positive energy in our meetings. Bob’s knowledge of the published literature, wisdom regarding clinical and biologic aspects of disease, and his sincere interest in advancing scientific discovery was motivation to us all. He had an innate ability to know what was the next “big idea” on the horizon. His optimism was contagious. Our scientific discussions were rich and interesting. Bob understood collaborative science and the importance of cross-disciplinary teams well before this became more the norm in science.

We had the privilege to co-author many scientific papers together. Dozens of trainees worked on one or more aspects of this work with Dr. Genco. OsteoPerio was just one part of the remarkable legacy Bob left. What a remarkable breadth of work he accomplished - it is hard to believe any one individual could produce so much, even knowing the pace at which he worked. However, his scientific productivity only reflects part of his story. It was Bob Genco, the person, the friend, the collaborator and the mentor we miss the most. He was a gentleman who was always kind and respectful with everyone he engaged. He had sincere interest in others - interest in their backgrounds, their families, and their own research pursuits. He shared stories of his own background, family members and interests. He was a thoughtful mentor to all the students we shared. He served as a role model to everyone he encountered. Bob was working at the highest level until the day he passed. Few in the field of oral biology and periodontal disease have not been influenced in some way by Bob Genco or his work. We are among them. Since Bob passed, our research meetings have continued, but our discussions are far less rich without Bob at the table. We miss him. It has truly been a blessing to have had the opportunity to collaborate with such a great talent, generous mentor, brilliant scientist and remarkable man. He was one of a kind.

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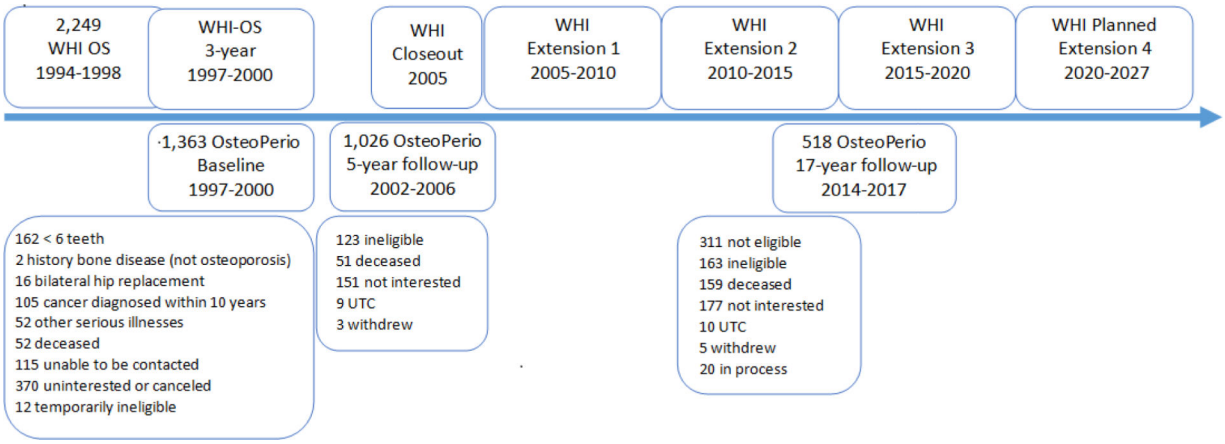


**Figure 1 -**

Flow chart of OsteoPerio participant enrollment and loss to follow-up.