Cohort profile: the Buffalo OsteoPerio microbiome prospective cohort study

Hailey R Banack,1 Robert J Genco,2 Michael J LaMonte,1 Amy E Millen,1 Michael J Buck,3 Yijun Sun,4 Christopher A Andrews,5 Kathleen M Hovey,1 Maria Tsompana,6 Daniel I McSkimming,7 Jiwei Zhao,8 Jean Wactawski-Wende,1 for the OsteoPerio Study Group

ABSTRACT

Purpose The Buffalo Osteoporosis and Periodontal Disease (OsteoPerio) study is a prospective cohort study focused on the relationship between the microbiome and oral and systemic health outcomes in postmenopausal women. The cohort was established to examine how the oral microbiome is affected by (and how it affects) periodontal disease presence, severity and progression and to characterise the relationship between the microbiome, lifestyle habits and systemic disease outcomes.

Participants Participants (n=1342) were postmenopausal women who were participating in the Women’s Health Initiative observational study at the Buffalo, New York clinical centre. There were 1026 participants at the 5-year follow-up visit and 518 at the 15-year visit.

Findings to date Data collected include questionnaires, anthropometric measures, serum blood and saliva samples. At each clinic visit, participants completed a comprehensive oral examination to measure oral health and the oral microbiome. Preliminary findings have contributed to our understanding of risk factors for periodontal disease and the relationship between the oral microbiome and periodontal disease.

Future plans The novel microbiome data collected on a large sample of participants at three time points will be used to answer a variety of research questions focused on temporal changes in the microbiome and the relationship between the oral microbiome and oral and systemic disease outcomes. Little is currently known about the relationship between the oral microbiome and health outcomes in older adults; data from the OsteoPerio cohort will fill this gap. Microbiome samples are currently being analysed using next-generation sequencing technology with an anticipated completion date of late 2018.

INTRODUCTION

The Buffalo Osteoporosis and Periodontal Disease (OsteoPerio) study is a prospective cohort study that was established to explore risk factors for the development and progression of osteoporosis and periodontal disease in postmenopausal women. As technology has advanced, the focus of the cohort has evolved from traditional risk factor epidemiology to a novel exploration of the entire oral microbiome and its relationship to health outcomes in postmenopausal women. This cohort will improve our understanding of the complex relationship between the oral microbiome and health and disease outcomes in older adults. It will also contribute to the growing body of literature focused on understanding the human microbiome. Up to this point, most studies of the microbiome have been descriptive reviews, cross-sectional or case-control studies rather than prospective studies with longitudinal follow-up.1

There has been a great deal of excitement about the potential of microbiome research in epidemiology and its application to clinical practice.1 Results from the Human Microbiome Project described the diversity, composition and function of the human microbiome, and recent calls have been made to further understand the role of the microbiome in disease aetiology.2 The microbiome is broadly defined as a collection of microbes living in and on our bodies, including bacteria, viruses and other organisms.1 Disruption of the microbiome is called dysbiosis, resulting in an imbalance between good and bad bacteria. Dysbiosis can result in an overabundance of bacteria, as is the case...
in periodontal disease, including the emergence of oral pathogens and reduction of commensal organisms. The concept of dysbiosis is central to understanding periodontal disease initiation and progression. Dysbiosis is thought to trigger immunoinflammatory processes that play a major role in the destruction of the soft tissues and bone supporting the teeth in the jaw. In a woman with osteoporosis, we would expect to see a greater reduction in oral bone and more severe periodontal disease, due to the combination of bacterial bone resorbing factors and lower local bone mineral density compared with a similarly aged woman without osteoporosis. There is scientific interest in understanding the factors that cause dysbiosis, as well as understanding the factors that restore a dysbiotic microbiome to a healthy state.

Technological advances have recently enabled the exploration of the human microbiome in large epidemiological cohorts. The samples collected from the OsteoPerio cohort will be analysed using innovative next-generation sequencing technology, including high-throughput DNA sequencing. Our objectives are to provide new information on the composition and diversity of the oral microbiome in older women; to examine how the oral microbiome is affected by and how it affects periodontal disease presence, severity and progression; and characterise the relationship between the microbiome and lifestyle habits and other factors (ie, smoking, diabetes, diet). Results from this cohort study will make substantive contributions to the field of microbiome research as well as methodological contributions by developing and implementing rigorous quality control procedures and statistical methods for analysing large metagenomic data.

COHORT DESCRIPTION

Study population and setting

The participants of the OsteoPerio cohort were recruited from participants in the Women’s Health Initiative (WHI) observational study (WHI-OS) at the Buffalo, New York clinical centre. The study was approved by the Institutional Review Board at the University at Buffalo. The OsteoPerio cohort is an ancillary study to the WHI, a major US research study of postmenopausal women, aimed at preventing morbidity and mortality in postmenopausal women. WHI participants from the Buffalo centre were recruited from 1993 to 1998. The OsteoPerio cohort was recruited approximately 3 years after WHI enrolment between 1997 and 2001. Study visits took place at baseline (1997–2001; n=1342), 5 years (2002–2005; n=1026) and 15 years (2014–2018; n=518). Figure 1 describes the flow of recruitment and retention of OsteoPerio participants. Table 1 describes demographic and anthropometric characteristics of the study cohort, stratified by visit (baseline, 5years, 15years).

Women who were enrolled in the WHI-OS who had fewer than six teeth, bilateral hip replacement, history of bone disease other than osteoporosis, history of cancer within previous 10 years or treatment for serious illness were ineligible for enrolment into the OsteoPerio study. At the 5-year follow-up, women were ineligible to participate if they had developed cancer or other immunosuppressive disorders, had cirrhosis or chronic hepatitis B or bilateral hip replacement in the interim since their baseline study visit. Additionally, there was a temporary restriction on women who had recently taken antibiotics, had recent diagnostic imaging involving contrast dye or had a dental cleaning in the past 30 days; they were ineligible to participate for the 30-day period after antibiotics/ imaging/dental visit but eligible thereafter. Women who had exposure to dental X-rays within the year prior to study visit were temporarily ineligible until at least a year had passed since their last dental X-ray. At the 15-year follow-up, the criteria remained the same with a slight modification for women who had been diagnosed with cancer: women currently undergoing treatment for invasive cancer were ineligible, but women were eligible to participate if 6 months had elapsed since their last cancer treatment.

Data collection and measures

Overview

Participants were scheduled for morning clinic appointments to allow for fasting blood measurements at each of their study visits. On the day of the visit, participants completed an informed consent form and study questionnaires. Blood sample and saliva collection, blood pressure measurements and anthropometric measurements were then completed. A comprehensive whole-mouth oral examination was performed by a dental examiner. Each component of the clinic visit is detailed below, and was repeated at baseline, year 5, and year 15.

Oral examination

Subgingival samples were taken from 12 index teeth or their substitutes using a paper point technique. Plaque was assessed at three surfaces per tooth. The paper point technique involved drying each tooth with cotton (to reduce cross contamination), placing absorbent paper points (Johnson & Johnson) into the gingival pocket of the mesiobuccal surface of the selected tooth for 10s, transferring the paper point to 4mL of Ringer’s solution, vortexing the samples to disperse the organisms, transferring the paper point to cryovials and frozen at −80°C directly after collection. Upper and lower arches from each of the three time points were pooled and transferred to cryovials for microbiome testing. Participants also provided 5mL of saliva, collected in a conical tube, over a 10min period. Cytokines and C reactive protein were measured in saliva.

The oral examination included examination of the head and neck, oral mucous membranes, recording of restorative appliances, as well as caries and missing teeth. Gingival assessment was done on three gingival sites per
tooth of all teeth present by scoring bleeding on probing. Calculus presence was assessed for each tooth on a three-point scale. Pocket depth, the distance in millimetres from the gingival margin of the tooth to the base of the sulcus/pocket, was measured on six surfaces per tooth of all the teeth present except the third molars using a constant force electronic periodontal probe (The Florida Probe System, Gainsville, FL, USA). The final component of the oral examination was oral radiographs (X-rays). Up to 11 standardised intraoral radiographs were taken from each participant and digitised; the number of radiographs taken depended on the number of teeth present.

**Blood measures**

Fasting blood samples were drawn by trained technicians using venipuncture, and then centrifuged, portioned to

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**Figure 1** Flow chart detailing participant enrolment and losses to follow-up in the Osteoporosis and Periodontal (OP) Disease study. *As of Jan 2018; temporary ineligibility was due to a variety of different reasons (ie, recent oral X-rays or radio luminescent dye tests). OS, observational study; WHI, Women’s Health Initiative; UTC, unable to contact.
serum, plasma and buffy coat, and frozen either in liquid nitrogen (baseline, year 5) or in −80°C freezers (year 15) in 0.5 mL aliquots. All samples are housed in the University at Buffalo’s Department of Epidemiology and Environmental Health biospecimen bank. Blood samples have been used to measure serum lipids, C reactive protein, blood glucose, blood insulin, comprehensive metabolic panel, reproductive hormones, 25-hydroxyvitamin D and several cytokines. Additional sample remains for future use in the biospecimen bank. An overview of laboratory variables already measured at each study visit is presented in table 2.

### Table 1 Characteristics of study participants at baseline, 5-year and 15-year follow-up visits

| Characteristic                              | Baseline | 5-year follow-up | 15-year follow-up |
|---------------------------------------------|----------|------------------|-------------------|
| **N**                                       | 1342     | 1026             | 518               |
| Age, at visit mean (SD)                     | 66.7 (7.0)| 70.4 (6.7)       | 79.9 (5.8)        |
| BMI (kg/m²), mean (SD)                      | 26.6 (5.1)| 26.7 (5.3)       | 27.2 (5.2)        |
| BMI N (%)                                   |          |                  |                   |
| Underweight/normal (≤24.9)                 | 590 (44.0)| 435 (42.4)       | 187 (36.1)        |
| Overweight (≥25.0–29.9)                    | 466 (34.7)| 378 (36.9)       | 208 (40.2)        |
| Obese (≥30.0)                               | 286 (21.3)| 212 (20.7)       | 123 (23.8)        |
| Missing                                     | 1        |                  |                   |
| Race N (%)                                  |          |                  |                   |
| American Indian/Alaska Native               | 6 (0.5)  | 5 (0.5)          | 4 (0.8)           |
| Asian/Pacific Islander                      | 4 (0.3)  | 3 (0.3)          | 3 (0.6)           |
| Black or African American                   | 22 (1.6) | 12 (1.2)         | 6 (1.2)           |
| Hispanic/Latino                             | 4 (0.3)  | 1 (0.1)          | 2 (0.4)           |
| White                                       | 1306 (97.3)| 1005 (98.0)     | 503 (97.1)        |
| Marital status, married N (%)               |          |                  |                   |
| Never married                               | 80 (6.0) | 58 (5.7)         | 30 (5.8)          |
| Divorced/separated                          | 120 (8.9)| 91 (8.9)         | 46 (8.9)          |
| Widowed                                     | 218 (16.2)| 146 (14.2)     | 52 (10.0)         |
| Presently married                           | 913 (68.0)| 720 (70.2)      | 385 (74.3)        |
| Marriage like relationship                  | 11 (0.8) | 11 (1.1)         | 5 (1.0)           |
| Education N (%)                             |          |                  |                   |
| High school                                 | 280 (21.2)| 213 (21.2)      | 94 (18.5)         |
| College                                     | 589 (44.6)| 438 (43.5)      | 213 (41.9)        |
| Post college                                | 452 (34.2)| 356 (35.4)      | 201 (39.6)        |
| Missing                                     | 21       | 19               | 10                |
| Cigarette smoking N (%)                     |          |                  |                   |
| Never                                       | 707 (52.7)| 555 (54.2)      | 281 (55.9)        |
| Former                                      | 590 (44.0)| 450 (44.0)      | 220 (43.7)        |
| Currently                                   | 44 (3.3) | 19 (1.9)         | 1 (0.2)           |
| Missing                                     | 1        | 2                | 16                |
| Alcohol consumption (ounces/day) mean (SD)  | 0.45 (0.7)| 0.51 (0.9)      | 0.53 (0.8)        |
| Periodontal disease (CDC/AAP) N (%)         |          |                  |                   |
| None                                        | 306 (23.1)| 333 (33.2)      | 123 (25.7)        |
| Mild                                        | 48 (3.6) | 45 (4.5)         | 14 (2.9)          |
| Moderate                                    | 759 (57.3)| 504 (50.2)      | 299 (62.6)        |
| Severe                                      | 211 (15.9)| 122 (12.1)      | 42 (8.8)          |
| Missing                                     | 18       | 22               | 40                |

AAP, American Academy of Periodontology; BMI, body mass index; CDC, Centers for Disease Control and Prevention.
Study questionnaires

Demographic information and personal characteristics were assessed via self-report questionnaires. Questionnaires collected information on demographic factors, medical and oral health history, dental hygiene, smoking, dietary intake (via Food Frequency Questionnaire), medications and dietary supplements and use of menopausal hormone therapy. As part of the larger WHI study, participants were asked questions about a wide range of medical, psychological and behavioural factors, health conditions and diseases. Annual assessments to update health outcomes are part of the WHI study.

Anthropometric measures

All measures were conducted by trained and certified staff according to standardised protocols. Height and weight were measured by a trained technician using a calibrated wall-fixed clinical stadiometer and weight scale. Waist circumference was measured at the narrowest part of the torso at end exhalation using an anthropometric tape measure. Systolic and diastolic blood pressure were measured after 5 min of quiet sitting with legs uncrossed using a manual sphygmomanometer. Body composition, including fat mass, lean mass and both total-body and site-specific bone mineral density, was assessed using dual energy X-ray absorptiometry (Hologic QDR-4500).

Periodontal disease measures

Periodontal disease presence and severity were defined using two definitions, one derived from the OsteoPerio study based on alveolar crestal height (ACH), a measure of oral bone loss, and tooth loss and the other from the Centers for Disease Control and Prevention/American Academy of Periodontology (CDC/AAP). The OsteoPerio definition categorised participants into none (whole-mouth mean ACH <2 mm, no tooth loss due to periodontal disease), mild/moderate (whole-mouth mean ACH ≥ 2 and<3 mm or one or more sites with ≥4 mm and no tooth loss due to periodontal disease) and severe (whole-mouth mean ACH ≥ 3 mm or more than two sites with ≥5 mm or ≥1 tooth loss due to periodontal disease) and severe (whole-mouth mean ACH ≥3 mm or more than two sites with ≥5 mm or ≥1 tooth loss due to periodontal disease). The CDC/AAP definition used none/mild (neither moderate nor severe disease), moderate (≥2 interproximal sites with clinical attachment loss (CAL) ≥4 mm, not on the same tooth, or ≥2 interproximal sites with CAL 5 mm, not on the same tooth) and severe (≥2interproximal sites with CAL ≥6 mm, not on the same tooth, and ≥1 interproximal site with pocket depth ≥5 mm). The actual measured values for each of the measures obtained are also available for each participant by tooth and as whole-mouth mean and worst site values.

Analysis of the oral microbiome

The composition and diversity of the oral subgingival microbiome were assessed by 16S ribosomal DNA
Findings to date

Traditional risk factors for periodontal disease

Results from the OsteoPerio study have supported the link between bone mineral density and periodontal disease. Compared with women with normal systemic bone mineral density T-score, women with osteoporosis are nearly twice as likely to have oral bone loss, measured by ACH (OR=1.90; 95% CI 1.19 to 3.05). The relationship between systemic bone mineral density and oral bone loss is greater in women over age 70 (OR=3.57; 95% CI 1.42 to 8.97) compared with women less than age 70 (OR=1.63; 95% CI 2.83). 10 CAL, a measure of periodontal disease severity, was inversely associated with bone mineral density in the OsteoPerio study. Other results from our cohort have demonstrated that cigarette smoking and indicators of periodontal disease severity are strong risk factors for incident tooth loss in older women. 

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Oral microbiome

There were differences in the microbial composition and diversity among OsteoPerio participants with and without periodontal disease (figure 2). Among 30 women included in a pilot analysis, 15 (figure 2; labelled N1-15 on x-axis) with no periodontal disease and 15 (N16-30) with disease, there were noticeable differences in the relative abundance of commensal and pathogenic periodontal bacteria. Women with no periodontitis (N1-15) have a greater relative abundance of organisms associated with periodontal health (represented by blue, green and yellow colours), whereas women with periodontal disease (N16-30) have a greater abundance of potential pathogenic microorganisms (represented by red and orange). The colours in the figure correspond with the Socransky classification for oral bacteria. Figure 2 also demonstrates the variation in the microbiome composition among women with the same periodontal status, for example, participants 1, 8 and 11 have no periodontal disease but were found to have pathogenic bacteria (red/orange bacteria), while participants 19, 21 and 26 have severe periodontal disease but an abundance of non-pathogenic bacteria (blue/yellow/green).

Strengths and limitations

The OsteoPerio cohort fills an important gap in our understanding of the relationship between the oral microbiome and disease outcomes. The prospective study design, cohort size and linkage with larger WHI study are key strengths. To the best of our knowledge, the OsteoPerio study is the first study able to answer questions about how the oral microbiome changes as women age, determine the relationship of the oral microbiome composition and periodontal disease initiation and progression, and explore the association between the microbiome and disease relevant to ageing. The use of
novel metagenomics technology is another strength as the data generated from this cohort lie at the intersection of epidemiology, microbiology, bioinformatics and biostatistics. Once 16S rDNA sequencing is complete, the data can be used to answer an extremely wide range of questions about the oral microbiome and disease outcomes. The main limitation of the cohort is that the participants are all postmenopausal women, who are predominantly white and well educated. Additionally, participants were required to have at least six teeth to participate in the study and most women had good oral hygiene. This may affect the study findings because those with very severe periodontal disease may have had teeth extracted (resulting in fewer than six teeth present in the mouth) and individuals with good oral hygiene may have a lower oral bacterial load than those who have poor oral hygiene.

We also learned some valuable lessons applicable to researchers interested in microbiome research. Most valuable, perhaps, is the importance of collaborating with investigators across disciplines when assembling cohort studies for human microbiome research. Collaboration between researchers with diverse areas of expertise (epidemiology, dentistry/oral biology, statistics, biochemistry and bioinformatics) has enabled this study to be successful. Another lesson learned is the high degree of technical knowledge required to conduct microbiome analyses, including developing standard operating procedures, quality control metrics, computational strategies and choosing reference databases. It took a very long time to develop and implement these procedures because the field of microbiome research is still an emerging discipline and there are few ‘best practice’ guidelines to follow.

**Patient and public involvement**

OsteoPerio participants were given the opportunity to provide feedback on study visits (ie, visits too short, too long). As well, after the baseline visit, we conducted a survey to ask study participants what they used the study results for; whether they shared the results with their physician or made any lifestyle changes. Study results and updates are continually shared with OsteoPerio participants through mailed newsletters and in-person meetings. The purpose of the in-person meetings is twofold: to disseminate study findings and provide participants with the opportunity to ask about their research interests and priorities.

**Author affiliations**

1Department of Epidemiology and Environmental Health, University at Buffalo, The State University of New York, Buffalo, New York, USA
2Departments of Oral Biology, and Microbiology and Immunology, and Center for Microbiome Research, University at Buffalo, The State University of New York, Buffalo, New York, USA
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Collaborators OsteoPerio Study Group.

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