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An evaluation of potential sources of systematic error in the estimation of periodontal disease associations

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DISSERTATION

AN EVALUATION OF POTENTIAL SOURCES OF SYSTEMATIC ERROR IN THE ESTIMATION OF PERIODONTAL DISEASE ASSOCIATIONS

by

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ABSTRACT

Aim: To investigate the effect of different definitions of periodontitis, using partial-mouth measurements instead of full-mouth ones, and unmeasured confounders on periodontitis' associations with diabetes and cardiovascular disease (CVD).

Methods: Adults 30-79 years with ≥1 or ≥2 teeth in the 2009-2014 National Health and Nutrition Examination Survey for study 1 or 2, respectively. For study 1: periodontitis was defined using different thresholds while for study 2: it was defined using the CDC/AAP 2007 categorical definition and continuous measures were estimated using mean clinical attachment loss (CAL), Estimates of periodontitis were derived based on the full-mouth protocol and three partial-mouth protocols (PMPs), including the Ramfjörd teeth, the Community Periodontal Index for Treatment Needs teeth, and the random half-mouth. Effects of exposure and outcome misclassification of periodontitis were evaluated in relation to diabetes. Diabetes and CVD were ascertained using self-report. Percent relative bias (%RB) was calculated by comparing the odds ratios/beta estimates obtained from the full-mouth and PMPs. Study 3 used the dental

longitudinal study to look at the effects of simulated unmeasured confounders on survival analysis in the periodontitis-diabetes/CVD and diabetes-periodontitis associations.

Results: For study 1: the effects of clinical severity on the odds ratios were association dependent. Clinical measures and extent did not depend on the association. For study 2: percent relative bias was generally less than 10% for the severe categories while it tended to exceed 10% for moderate categories. Mean clinical attachment loss resulted in minimal bias. For study 3: presence of one source of unmeasured confounding (one confounder) showed that the diabetes-periodontitis association was robust to it unlike the periodontitis-diabetes/CVD associations.

Conclusion: These sources played a role in the periodontitis-diabetes, periodontitis-CVD, and diabetes-periodontitis associations. The associations were affected differently by each source. Some of these sources of systematic errors may change the conclusions of the associations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1: Introduction

Periodontitis etiology and associations with diabetes and cardiovascular disease

The American Academy of Periodontology (AAP) defines periodontitis as an "Inflammation of the periodontal tissues resulting in clinical attachment loss, alveolar bone loss, and periodontal pocketing" (1). One of the outcomes of periodontitis is tooth loss (2). The prevalence of periodontitis in the US population among those who were 30 years old or older according to the National Health and Nutrition Examination Survey was 46% in 2009-2012 and 42% in 2009-2014 (3, 4).

One of the risk factors associated with periodontitis is diabetes (5-7). Diabetics have three times the risk of developing periodontitis diagnosis compared to non-diabetics (8). Periodontitis is common among diabetics (9, 10). The causal mechanisms of both diseases are similar in that both are affected by inflammatory mediators or markers (11). These mediators include interleukins (IL-1β, IL-6), prostaglandin E2, Tumor necrosis factor-α (TNF-α), receptor activator of nuclear factor KB ligand (RANKL), matrix metalloproteinases (MMP-8, MMP-9, MMP-13), T cell regulatory cytokines (IL-12, IL-18), chemokines, and the inflammation marker C-reactive protein (12-17). Inflammatory markers such as C-reactive protein would lead to insulin resistance (18, 19). Both diseases start a cascade of inflammatory mediators mentioned above and in turn, affect each other (11). Decreased polymorphonuclear leucocyte (neutrophil) activity in both diseases takes place, leading to further inflammation and destruction (20-23). Periodontal treatment has been shown to reduce inflammatory markers among diabetics and non-diabetics (17, 24-27). This, in turn, may improve metabolic control of diabetics (11).

Another potential systemic disease that periodontitis is associated with is cardiovascular disease. According to CDC, cardiovascular diseases are a variety of conditions that affect the

heart including: coronary heart disease, heart attack (myocardial infarction), angina, aortic aneurysm and dissection, arrhythmias, atherosclerosis, atrial fibrillation, cardiomyopathy, congenital heart defects, heart failure, peripheral artery disease, and rheumatic heart disease (28). The association between periodontitis and CVD was explored in the literature and was inconsistent (29-34). Possible causal mechanisms could be that chronic periodontitis leads to systemic inflammation through inflammatory mediators, which raises markers of inflammation and in turn, stimulate vascular thrombosis (35-37). Another proposed causal pathway for the periodontitis-CVD association is through periodontal pathogens (P. gingivallis, B. forsythus, P. intermedia, and A. actinomycetemcomitans) which could induce thrombosis through activation of platelets (38-45). Another suggested causal pathway for periodontal pathogens may be through aiding the formation of coronary atherosclerotic plaques (46, 47). A recent meta-analysis showed that periodontal therapy had little or insufficient evidence of preventing CVD (48).

Several sources of errors may be present in the observed periodontitis- systemic disease associations. First, the case definition of periodontitis can also result in misclassification and change the strength of associations between periodontitis and diabetes and cardiovascular disease. Second, when using partial-mouth measurements instead of full-mouth measurement, misclassification bias may result. Third, unmeasured and/or uncontrolled confounding can partially or totally explain away the associations between periodontitis and diabetes and cardiovascular disease. The aim of this dissertation was to evaluate the three potential sources for the periodontitis' association with diabetes and cardiovascular disease. Study 1 looked into the role of misclassification of periodontitis as a result of case definitions on associations with diabetes and cardiovascular disease. Study 2 looked into the role of misclassification as a result of partial-mouth measurements of periodontitis on associations with diabetes and cardiovascular

disease. Study 3 looked into the role of unmeasured common causes (confounders) on associations with diabetes and cardiovascular disease. Studies 1 & 2 looked into a systematic error called misclassification bias while study 3 looked into another type of systematic error called confounding.

Overview of disease classification as a source of misclassification bias

Explanation of misclassification bias

Measurement bias (also known as information bias or misclassification bias). Given that diagnosis is a continuous phenomenon, misclassification can occur when measures of diagnosis are categorical. Such a categorization is applied to diseases or conditions of interest. For categorization to happen, a certain threshold value or cut-off-point is used to establish whether a person is diseased/non-diseased or exposed/non-exposed.

Sensitivity is defined as the percentage of people that are classified as diseased among those who are in truth diseases. Specificity is defined as the percentage of people that are classified as not diseased among those who in truth do not have it (49). The sensitivity and specificity of a test used to classify a population will differ according to the characteristics of that population (50).

Measures such as sensitivity and specificity are used to assess misclassification. Misclassification of a variable (e.g., smoking) arises when a person who smokes is incorrectly classified as not a smoker or vice versa. The impact of misclassification depends on what type of variable it occurs in (exposure, outcome, or covariate) (49, 50). The next paragraphs explain the concepts and mechanisms of misclassification of exposure or outcome.

Exposure misclassification

Exposure misclassification happens whenever people with a certain exposure (e.g., diabetes) are classified as non-diabetic (i.e., imperfect sensitivity) or non-diabetics are classified as diabetics (i.e., imperfect specificity). Non-differential exposure misclassification bias occurs when sensitivity or specificity is less than 100%, and the probability of misclassification is the same for all categories of a disease of interest (e.g., periodontitis) (left side of figure 1, at the end of this chapter) (49, 50). To clarify this, suppose a researcher again is interested in studying the association between diabetes as a binary exposure (yes/no) and periodontitis as a binary outcome (yes/no). Non-differential misclassification bias of the exposure (diabetes) means that sensitivity and specificity of diabetes are the same for periodontitis groups (with and without periodontitis). Framed in another way, the extent to which diabetics are classified as non-diabetic and vice versa is the same for people with and without periodontitis.

Differential exposure misclassification bias occurs when sensitivity or specificity is less than 100%, and the probability of misclassification is not the same for all categories of disease (diabetes in this case) (49, 50). This translates to sensitivity and specificity of diabetes not being the same for people with and without periodontitis (i.e., the extent to which people with diabetes are classified as not having it and vice versa is not the same for periodontitis groups). It is likely to happen once exposure (diabetes) is ascertained after data on the disease (periodontitis) are collected as the case in interviews where people with periodontitis may remember their diabetes status differently compared to ones without periodontitis (recall bias) or when interviewers are not blinded to the periodontitis status of the subjects and probe subjects with and without periodontitis differently to know their exposure (diabetes) (51, 52).

Exposure misclassification can affect the measure of association depending on whether it is non-differential or differential (49, 50). Non-differential exposure misclassification can be expected to result in a bias towards the null effect on average with deviations from the expectation that may exist (49, 50, 53-60). A polytomous exposure (has more than two categories), can bias the results away from or towards the null (50, 61-63). Differential exposure misclassification can produce bias in either direction (49, 50, 64).

Outcome misclassification

Misclassification of an outcome takes place whenever people with a certain disease (e.g., periodontitis) are classified as not having periodontitis (i.e., imperfect sensitivity) or people without periodontitis are classified as having periodontitis (i.e., imperfect specificity). The bias resulting from outcome misclassification can be either non-differential or differential. Non-differential outcome misclassification bias occurs when sensitivity or specificity is less than 100%, and the probability of misclassification is the same for all categories of exposure of interest (e.g., diabetes) (right side of figure 1, at the end of this chapter) (49, 50). To elaborate on this, suppose a researcher is interested in studying the association between diabetes as a binary exposure (yes/no) and periodontitis as a binary outcome (yes/no). If there is non-differential misclassification bias of the outcome (periodontitis), it means that sensitivity and specificity of periodontitis are the same for diabetics and non-diabetics. To put it in other words, the extent to which people with periodontitis are classified as not having periodontitis and vice versa is the same for both groups of diabetes (diabetics, non-diabetics).

Differential outcome misclassification bias is when sensitivity or specificity is less than 100%, and the probability of misclassification is not the same for all categories of exposure (diabetes) (49, 50). To keep up with the above example, there would be differential outcome

misclassification bias of periodontitis when sensitivity and specificity of periodontitis are not the same for diabetics and non-diabetics. Differential misclassification is likely to occur when periodontitis is detected more among diabetics because health care providers are more likely to screen their mouths compared to non-diabetics (medical surveillance or detection bias) or when diabetics are more likely to report or answer correctly when asked about their periodontitis status than non-diabetics or vice versa (respondent bias) (52).

Like exposure misclassification, outcome misclassification can affect the validity of the measure of association. The magnitude and direction of this change depend on whether the outcome misclassification is non-differential or differential (49, 50). Non-differential outcome misclassification can be expected to result in a bias towards the null effect on average with deviations from the expectation that may occur (49, 50, 53-60). An exception to non-differential outcome misclassification biasing results towards the null is if the outcome has imperfect sensitivity (<100%) and perfect specificity (100%). In this case, the risk ratio would not be affected while the risk difference, odds ratio, and rate ratio will be biased towards the null. However, this comes with a cost of decreased precision for the risk ratio (50, 65). If the risk of a disease is low (<10%), then the odds and rate ratios will be biased to a lesser extent (49). Differential outcome misclassification on the other hand, is unpredictable (that is it can bias the measure towards the null or away from it) (49, 50, 64).

Periodontitis classification

Classification of the periodontal condition in terms of disease state has long been a debated issue. However, to address the lack of an accepted case definition, and primarily for surveillance efforts, case definitions were developed recently, with growing acceptance by the scientific community. These definitions are often tooth-based, incorporating the number of teeth

with varying severity of Clinical Attachment Loss (CAL) and Probing Pocket Depth (PD), to diagnose periodontitis and categorize it according to severity and extent (66). The most commonly used tooth-based definitions include: 1) the definitions originally proposed in 2007 by the Centers for Disease Control and Prevention (CDC) in collaboration with American Academy of Periodontology (CDC/AAP) and updated in 2012, and 2) those proposed in 1999 by the International Workshop on Classification of Periodontal Diseases, and updated in the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions (67-69). The CDC/AAP definition was originally developed for surveillance purposes and may lack relevance to biologic or clinical research (68). This definition uses both probing pocket depth (PD) and clinical attachment loss (CAL) to categorize periodontitis. The recent classifications and definitions proposed by the 2017 World Workshop aim to distinguish between different forms, or phenotypes, of the periodontal condition and thus include classifications for disease severity, complexity, and extent, as well as "grade" to indicate progression. The aim of this classification system was to provide a system of case definitions that could be applied across multiple domains, including surveillance, clinical practice, and research. In this system, classifications focus primarily on CAL to determine severity, but also incorporate alveolar bone loss and tooth loss. Whereas, probing depth is applied to determine complexity.

Regardless of initial intention, both approaches to disease classification are currently used to estimate both exposure effects of periodontitis on the risk of systemic conditions, as well as to measure periodontitis etiologies, with the CDC/AAP definition rapidly gaining wide appeal for use in the epidemiologic study of periodontitis. For simplicity as a means of identifying mechanisms of bias in the estimation of periodontitis disease associations, this dissertation project applies only the 2007 CDC/AAP definition. Specifically, the measured association

between periodontitis and diabetes and cardiovascular disease could be affected by misclassification bias as a result of classification based on the number of teeth and severity thresholds of clinical measures that do not adequately capture the disease state of relevance in the association under study. The CDC/AAP definition uses a single cut-off point of CAL, PD, or both and a single cut-off point of the number of teeth with a certain level of CAL, PD, or both to ascertain periodontitis. These cut-off points were not informed by risk thresholds for specific associations and therefore may reflect misclassification. Different thresholds or cut-off points to define periodontitis may affect the strength of association between periodontitis as an exposure and diabetes and cardiovascular disease. When periodontitis is an outcome, as in systemic disease associations with periodontitis, the mechanism of misclassification of periodontitis as a result of different case definitions is different than when periodontitis is exposure and so is the effect on the strength of association. In this case, the cut-off points should not be informed by risk thresholds for associations with systemic disease. Study 1/Chapter 2 presents a study aimed at determining the presence of risk thresholds of diabetes and cardiovascular disease for periodontitis via different classifications of periodontitis as well as how these classifications affect diabetes association with periodontitis.

Full-mouth measurements of probing pocket depth (PD) and clinical attachment loss (CAL) are the gold standard for ascertaining periodontitis (i.e., measuring all six sites of all 28 teeth, excluding third molars) (70-73). However, full-mouth measurements are time-consuming and costly (70). Another source of misclassification is introduced through the use of partial-mouth protocols, which compounds the misclassification introduced through disease classification. The impact of using partial-mouth measurements on estimates of periodontitis' prevalence and severity has previously been assessed (70-73). Partial-mouth measurement will

consistently underestimate the prevalence of periodontitis (73). Under tooth-based-categorical-definitions, partial-mouth measurement cannot detect periodontitis if it was not detected in full-mouth measurement; That is, partial-mouth techniques cannot have false positive results and therefore, have a specificity of 100% (73). However, if mean CAL (a continuous measure) alone is used to diagnose periodontitis instead of tooth-based-categorical-definitions, it is possible for a partial-mouth protocol to have a higher mean CAL than that obtained from full-mouth measurement.

The mechanism of periodontitis misclassification depends on whether it is ascertained as an exposure or an outcome (49). Therefore, the potential for bias due to partial-mouth measurement may differ based on whether periodontitis is exposure or an outcome. Study 2/Chapter 3 presents a study aimed at investigating the impact of periodontitis misclassification resulting from use of several partial mouth protocols on its association as an exposure with diabetes and cardiovascular disease and as an outcome with diabetes in relation to full-mouth measurements.

Overview of confounding as a source of bias

Confounding is the presence of a variable (confounder) that is a common cause of both an exposure and an outcome and not in the causal pathway from the exposure to the outcome (i.e., not an intermediate) that causes mixing of effects with the exposure on the outcome (i.e., explains the association between the exposure and the outcome) (64).

Causal diagrams or directed acyclic graphs (DAGs) is a method proposed by Judea Pearl to select the confounders and appropriately control for confounding in an association (74). Figures 2 and 3 (at the end of this chapter) depict the hypothesized causal associations between

periodontitis and diabetes and cardiovascular disease, respectively with their confounders from the literature using DAGitty (75) and modified by Inkscape (76).

In figure 2, the arrow from stress to smoking means that stress cause smoking (i.e., knowing the stress status of participants, determines the smoking status on average). If two variables are not connected by an arrow, then they are independent. The pathway $diabetes \rightarrow periodontitis$ is the causal pathway of interest. There are other non-causal pathways connecting diabetes and periodontitis such as $diabetes \leftarrow age \rightarrow periodontitis$ (i.e., diabetes and periodontitis will be expected to be associated because they have a common cause, age). The pathway $diabetes \leftarrow age \rightarrow periodontitis$ is called an open back-door pathway because information can be traced from periodontitis back to diabetes. On the other hand, the non-causal pathway $diabetes \leftarrow stress \rightarrow hypertension \leftarrow age \rightarrow periodontitis$ is called a closed back-door pathway because it is blocked at hypertension (notice the arrows coming into hypertension from both sides). If age is controlled, then the non-causal pathway $diabetes \leftarrow age \rightarrow periodontitis$ will be blocked.

The goal of causal diagrams is to select the sets of variables that eliminate confounding through a process known as back-door path analysis (i.e., control all variables that close all back-door paths from periodontitis to diabetes so that only the causal pathway $diabetes \rightarrow periodontitis$ is left open). DAGitty can detect the sets of variables (known as minimally sufficient sets) that fulfill closing all back-door paths between diabetes and periodontitis to control for confounding. More can be known about DAGs here (74, 77, 78). The minimally sufficient set of variables to control for in the periodontitis-diabetes/diabetes-periodontitis association based on the causal diagram below were age, gender, smoking, obesity, and

race/ethnicity. For the periodontitis-CVD association, the minimally sufficient set of variables were age, gender, smoking, obesity, race/ethnicity, and diabetes.

If there was a variable in the literature that is not yet known to be a common cause of both periodontitis and systemic disease and not in the causal pathway, it could affect cause remaining (residual) confounding even after appropriately adjusting for all selected confounders. Thus the presence of an unknown confounder can still explain away the associations between periodontitis and diabetes and cardiovascular disease. Study 3/Chapter 4 present a study that aimed to investigate the effect of an unknown unmeasured confounder on the association between periodontitis and diabetes and cardiovascular disease.

Common data sources used to explore sources of bias

The National Health and Nutrition Examination Survey [NHANES] is a national surveillance program designed to monitor the health and nutrition status of children and adults living in the United States. Initially implemented in 1971, this program administers a survey and conducts examinations and provides a nationally representative sample of the US non-institutionalized civilian population. The NHANES began including self-report items for dental health in 1971 and implemented partial-mouth detailed periodontal exams in 1999 for purposes of estimating the prevalence of periodontitis. More recently, detailed full-mouth periodontal exams were implemented in 2009 to validate these prior estimates. During the cycles in which full-mouth clinical exams were conducted, adults aged 30 years and above went through a safety exclusion questionnaire at home interviews and also prior to examination. Those who had a heart transplant, artificial valve, heart disease at birth (not including mitral valve prolapse), bacterial endocarditis, had been told by their doctors they had (rheumatic fever, hemophilia, kidney

disease, requiring renal dialysis, a pacemaker or automatic defibrillator, or a hip bone/joint replacement) were excluded from periodontal examination

Studies 1 and 2 utilize data from the NHANES 2009-2014 study to explore misclassification of periodontitis by case definitions and partial-mouth measurements, respectively, on periodontitis association with diabetes and cardiovascular disease. Adults aged 30 to 79 years, who had at least one permanent tooth present during oral examination (study 1) / two permanent teeth (study 2), and had complete periodontal examination were eligible.

Periodontitis ascertainment

In NHANES 2009-2014, trained and calibrated dental hygienists (2009-2010) and dentists (2011-2014) carried out full-mouth periodontal examinations of the participants with six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) per tooth measured for PD and gingival recession. PD was defined as the distance between free gingival margin to the base of the sulcus. Gingival recession was defined as the distance between the free gingival margin and the cemento-enamel junction. Both PD and gingival recession were measured in mm with a periodontal probe. CAL was calculated as PD minus the gingival recession.

Only permanent teeth and permanent root fragments that had a CEJ and part of the clinical crown were measured for PD and CAL. If the natural tooth was missing, had orthodontic banding, was splinted, was hemisected which interfered with probing, had heavy calculus that interfered with probing, did not have a clear cemento-enamel junction, or had no visible clinical crown, PD and CAL were scored as could not be assessed (79).

Numbers of teeth present were calculated from permanent teeth and any permanent root fragments. Periodontitis was defined using different cut-off points of the number of teeth,

the severity of CAL, and PD using measurement on four interproximal sites per tooth (mesio-buccal, mesio-lingual, disto-buccal, and disto-lingual) for all 28 teeth (third molar excluded because there were no periodontal measurements for them in NHANES). Interproximal sites , because they reduce how gingival recession affects the accuracy of PD (i.e., exclude attachment loss related to reasons other than inflammation) (80, 81).

Ascertainment of systemic conditions of interest – diabetes and cardiovascular disease

Participants in NHANES were asked during home interviews whether they have ever been told by their health professionals that they had diabetes (yes, no, or borderline) and coronary heart disease, angina, heart attack, or stroke (yes, no). In this study, we dichotomized self-reports of ever being diagnosed with diabetes (yes / no or borderline) and cardiovascular disease status (yes / no). Those who refused or did not know the answer had their diabetic status set to missing. Those who refused or did not know the answer, or did not answer yes to any of the conditions and had at least one condition missing had their cardiovascular disease status set to missing.

Ascertainment of Demographics and behaviors

Age at doorstep screening for NHANES was asked in all cycles (2009-2014). However, ages above 80 years old were not documented and instead categorized as ≥ 80. Therefore, in these studies, age at screening was used and analyzed as a continuous variable. Race/ethnicity was categorized as Mexican American, other Hispanic, Non-Hispanic White, Non-Hispanic Black, and other race including multi-racial. In this study, Mexican American and other Hispanic were combined in one category.

Data included if participants smoked at least 100 cigarettes in their lifetime, age when they started smoking regularly, whether they still smoked at the time of the home interviews, age

when they last smoked cigarettes regularly, number of usual cigarettes smoked in a day at the time they quit, number of days smoked with past 30 days of the home interview and average cigarettes per day during past 30 days (82).

In these studies, subjects who never smoked 100 cigarettes in their lifetime or never regularly smoked were considered as never smokers. Participants who smoked at least 100 cigarettes in their lifetime, ever smoked regularly, and were not smoking at the time of the home interview were considered as past smokers. Subjects who were still smoking at the time of the interview were considered current smokers.

Duration of smoking in years was calculated as the difference between the age when subjects last smoked cigarettes regularly and age when they first started smoking regularly for past smokers. For current smokers, it was the difference between their age at the time of interview and their age when they first started smoking regularly. The smoking intensity was calculated as the average number of cigarettes smoked in a day at the time past smokers quit smoking. For current smokers, smoking intensity was calculated as the average number of cigarettes smoked in a day for the past 30 days of the interview. Pack-years of cigarettes was calculated as (average number of cigarettes smoked in a day multiplied by the number of years smoking) divided by 20. Pack-years of cigarettes was used to control for smoking. If one of the above smoking questions had a missing value, then pack-years was set to missing.

Obesity was calculated from standing height and waist circumference, which were measured in cm (83). We converted these measurements to meters and computed waist to height ratio as a proxy for obesity and was analyzed as a continuous variable.

Figures

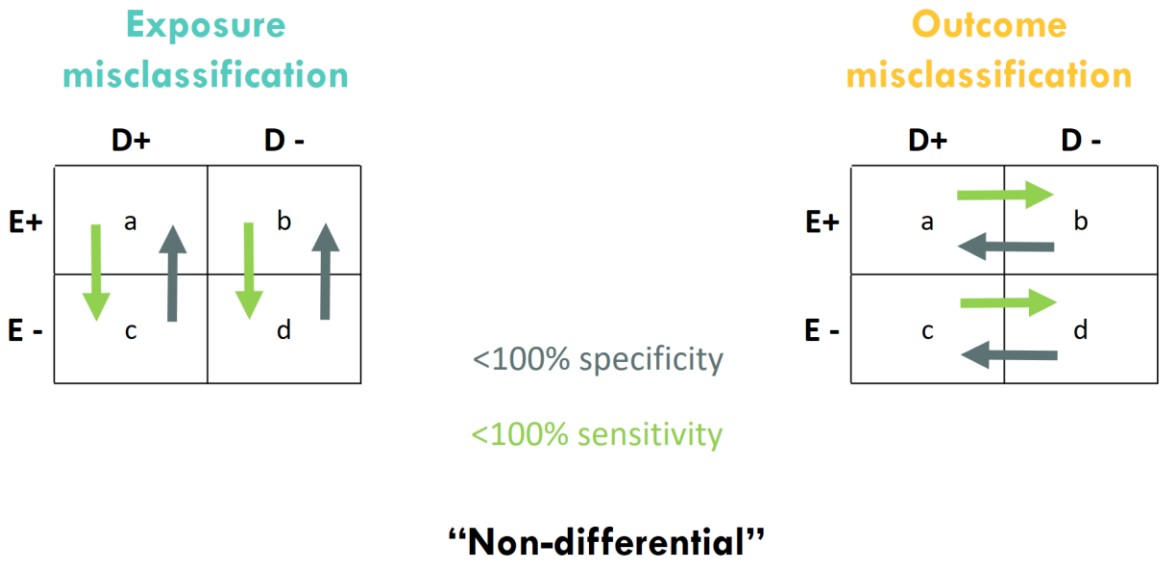

Figure 1. Explanation of non-differential misclassification bias.

E: exposure, **D:** disease.

Left side: exposed people are not completely classified correctly due to imperfect sensitivity and thus some would be misclassified as having no exposure when in fact they had the exposure. On the other hand, non-exposed are not completely classified correctly and thus some would be misclassified as having the exposure when in fact they did not. This misclassification is the same for the diseased and non-diseased evidenced by the same width of arrows.

Right side: diseased people are not completely classified correctly due to imperfect sensitivity and thus some would be misclassified as having no disease when in fact they had the disease. On the other hand, non-diseased are not completely classified correctly and thus some would be misclassified as having the disease when in fact they did not. This misclassification is the same for the exposed and non-exposed evidenced by the same width of arrows.

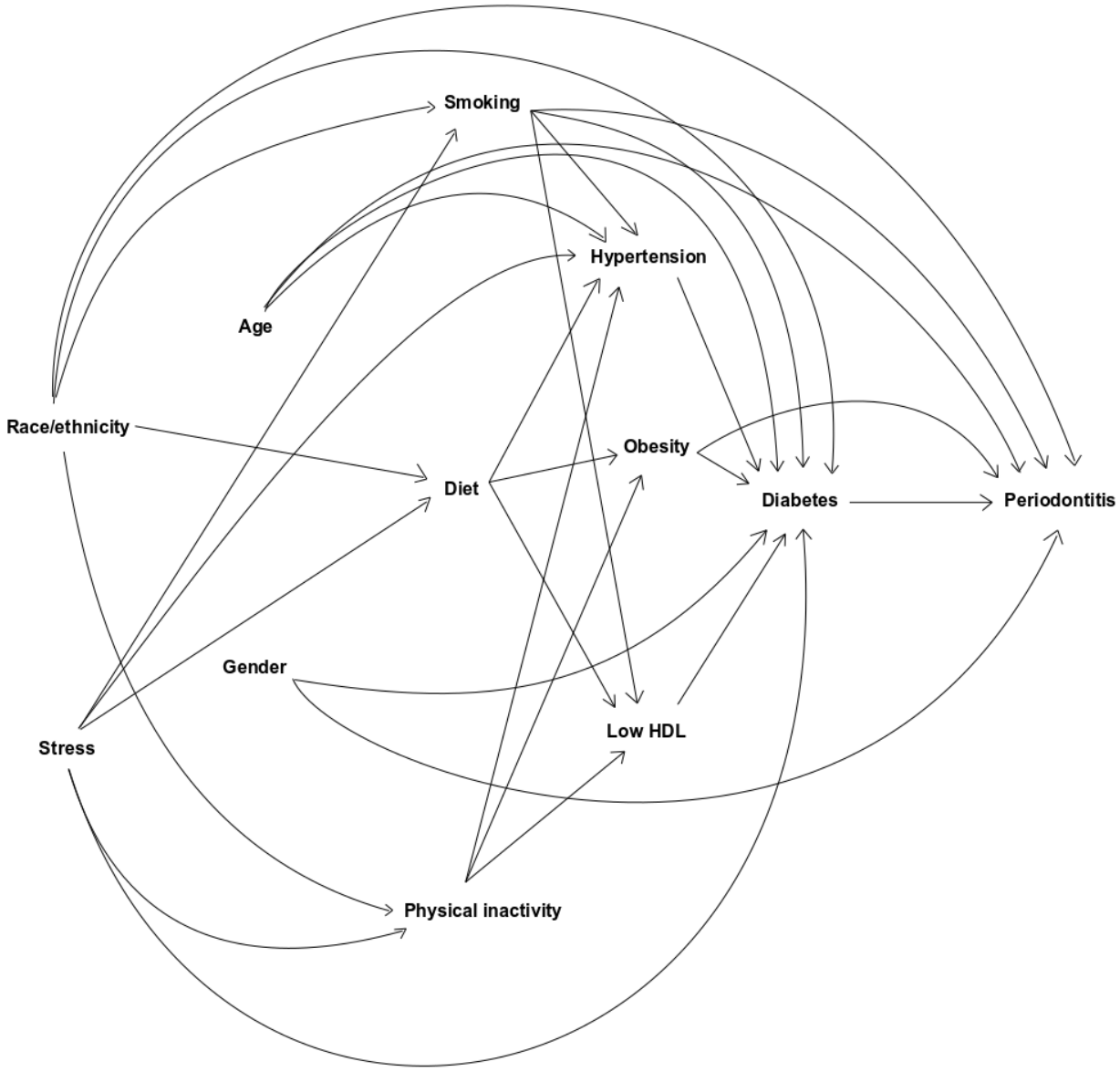

Figure 2. Directed acyclic graph of the periodontitis-diabetes/diabetes-periodontitis associations.

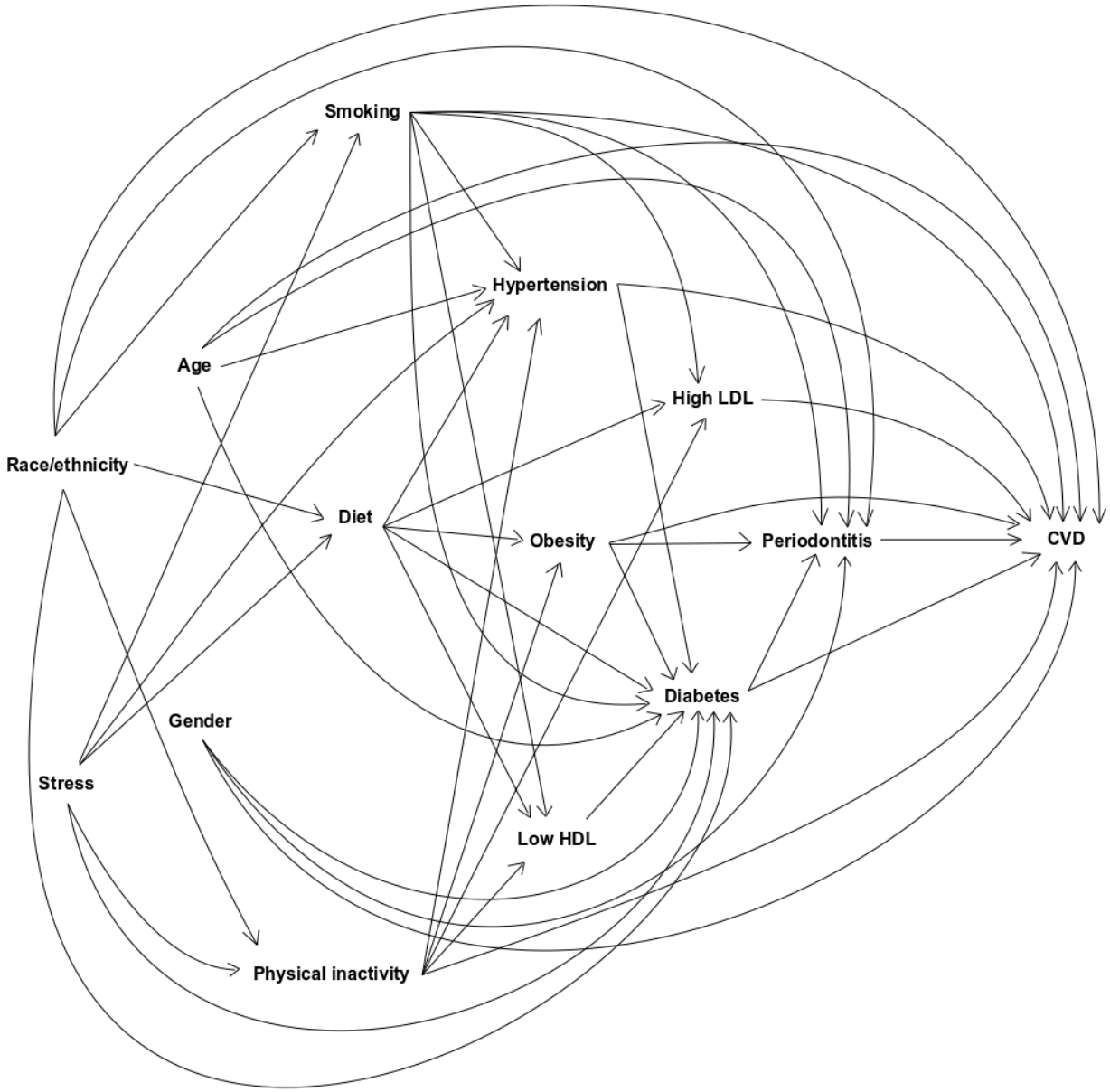

Figure 3. Directed acyclic graph of the periodontitis-CVD association.

Chapter 2: Study 1

The Impact of Tooth-based Clinical Thresholds of Periodontitis Classification in Associations with Diabetes and Cardiovascular Disease

Introduction

The measured association between periodontitis and diabetes and cardiovascular disease could be affected by misclassification bias as a result of classification based on the number of teeth and severity thresholds of clinical measures that do not adequately capture the disease state of relevance in the association under study. For example, the decision to treat hypertension may be based on cardiovascular and coronary heart diseases estimation (84). The CDC/AAP definition uses a single cut-off point of CAL, PD, or both and a single cut-off point of the number of teeth with a certain level of CAL, PD, or both to ascertain periodontitis. These cut-off points were not informed by risk thresholds for specific associations and therefore may reflect misclassification. Different thresholds or cut-off points to define periodontitis may affect the strength of association between periodontitis as an exposure and diabetes and cardiovascular disease. However, the effect on the association when periodontitis is an outcome may result in underestimation of the effect, if the misclassification is non-differential, i.e., not based on the exposure status.

The aim of this paper was to study the impact of using different cut-off points for the required number of teeth with a certain level of CAL or PD, as well as the cut-off points for clinical severity on periodontitis associations with diabetes and cardiovascular disease. Specifically, investigate the presence of risk thresholds of diabetes and cardiovascular disease for periodontitis via different classifications of periodontitis as well as how these classifications

affect diabetes association with periodontitis. To accomplish these aims, we used data from the National Health and Nutrition Examination Survey (NHANES) 2009-2014.

Methods

We used data from NHANES 2009-2014. The 2009-2014 cycles were the only cycles with full-mouth measurements of CAL and PD on all six sites of each tooth. Thus, it allowed defining periodontitis in multiple ways. Numbers of teeth present were calculated from permanent teeth and any permanent root fragments. Periodontitis was defined using different cut-off points of number of teeth, severity of CAL, and PD using measurement on four interproximal sites per tooth (mesio-buccal, mesio-lingual, disto-buccal, and disto-lingual) for all 28 teeth (third molar excluded because there were no periodontal measurements for them in NHANES) (80, 81). Table 1 shows the definitions that were used for periodontitis in this analysis. Periodontitis was analyzed as a binary (yes/no) variable according to the different definitions.

Statistical analysis

Multiple independent analyses were run to evaluate the role of periodontitis classification in periodontitis disease associations when periodontitis was the exposure of interest, i.e. exposure misclassification, and when periodontitis was the outcome of interest, i.e. outcome misclassification. In all cases, misclassification was assumed to be non-differential. All statistical analysis was carried out using SAS 9.4. Complete case analysis was conducted.

To evaluate exposure misclassification, multivariable binary logistic regression models were separately constructed for each outcome, i.e. diabetes and cardiovascular disease. The periodontitis exposure was evaluated as a binary variable, classified according to each definition of periodontitis (see Table 1). Similarly, evaluations of outcome misclassification were

conducted by constructing multivariable binary logistic regression models where the effects of diabetes as a categorical variable were estimated for each binary periodontitis outcome.

For all models, odds ratios and 95% CI were reported. The regression models were conducted using proc survey options in SAS, taking into account the complex design of NHANES and exam weight. All models were adjusted for potential confounders. Figures 1 and 2 show the hypothesized causal diagrams of the periodontitis-diabetes and periodontitis-CVD associations, respectively. The minimally sufficient set of variables to control for in the periodontitis-diabetes/diabetes-periodontitis association based on the causal diagram below was age, gender, smoking, obesity, and race/ethnicity. For the periodontitis-CVD association, the minimally sufficient set of variables was age, gender, smoking, obesity, race/ethnicity, and diabetes. No model selection procedures were conducted.

Stratified analysis

To assess the potential impact of varying numbers of teeth across participants, we estimated associations among those with less than or equal to 15 and among those with more than 15 teeth. This stratified analysis attempts to identify the potential impact of prevalent cohort bias, a special form of left truncation or selection bias, in which subjects who already had periodontitis and lost their teeth due to periodontitis may either not make it into the analytic cohort, or may be misclassified as a non-case of periodontitis (85).

Results

The sample eligible for this analysis was 10,147. Table 2 shows the distribution of the potential confounders, diabetes, cardiovascular disease, and the number of teeth of the overall sample. Almost half of the sample were males and the majority were non-Hispanic whites. The

mean number of teeth present during examination and the mean number of teeth that were actually measured for both CAL and PD were not that different (24 vs. 23, respectively).

With complete case analysis for the periodontitis-diabetes/diabetes-periodontitis associations, the sample was 9,593 while it was 9,563 for the periodontitis-cardiovascular disease. Joint distributions were evaluated and determined that sufficient sample size was available, and all regression models converged.

Periodontitis as an exposure

Table 3 shows the different associations of periodontitis with diabetes when the periodontitis exposure was defined using different cut-off points or thresholds in adjusted multivariable logistic regressions. The odds ratio of having diabetes for participants with periodontitis compared to those without periodontitis, at all thresholds, were in the positive side (i.e., > 1) on average. The definitions of periodontitis resulted in widths of the 95% intervals (i.e., defined as the upper bound divided by the lower bound for an odds ratio measure) ranging from (1.4 – 3.0). Most of the 95% confidence interval were compatible with lower a bound around 0.8 or 0.9 and were compatible with more positive values (i.e., > 1) than negative (i.e., < 1).

When severity (i.e., cut-off point in mm) and extent (i.e., number of teeth) were held fixed, using CAL only resulted in higher odds of diabetes than when using PD only. When either CAL or PD were used to define periodontitis, the odds ratios were similar to the odds ratio when periodontitis was defined using CAL only, suggesting that CAL is what drove the measure of association rather than PD. When both CAL and PD were used to define periodontitis, the odds ratios were generally closer to the estimates when PD only than when CAL only was used. This

suggests that PD is what drove the measure of association when both CAL and PD were required.

When the severity was fixed and CAL was only used, there was a decrease in the odds ratio as the definition became more stringent in terms of the number of teeth. When the extent was fixed and CAL was only used, there was a decrease in the odds ratio as the definition became more stringent in terms of clinical severity in mm. The results applied to when PD was only used too.

When results were stratified by the number of teeth present (Table 4), the odds ratios tended to be on the positive side (i.e., harmful) in both strata. Participants who had 1-15 teeth had a range of odds ratio (0.67 – 3.61) while those with > 15 teeth had a narrower range of odds ratios (0.74 – 1.36). The difference in odds ratio between using CAL only and PD only was even more accentuated among participants with 1-15 teeth while the differences were minimized among those with >15 teeth. The patterns based on severity and extent were even stronger among those with 1-15 teeth.

Table 5 shows the different associations of periodontitis as an exposure with cardiovascular disease as an outcome when periodontitis was defined using different cut-off points or thresholds in adjusted multivariable logistic regressions. The odds ratio of having cardiovascular disease for participants with periodontitis, at all thresholds, compared to those without periodontitis majorly resulted in odds ratio higher than 1.10. The definitions of periodontitis resulted in widths of the 95% intervals ranging from (1.5 – 7.3). Most of the 95% confidence interval were compatible with lower a bound around 0.8-1.0 and were compatible with more positive values (i.e., > 1) than negative (i.e., < 1).

The periodontitis-CVD association had similar patterns to the periodontitis-diabetes association in terms of changing clinical parameters and the number of teeth, but there was no pattern when severity was changed. Stratified analysis (Table 6) showed the same results as in the periodontitis-diabetes association but the difference was that participants with 1-15 teeth had odds ratios (0.24 – 1.38) that were mostly protective while those with > 15 teeth had a narrower range (0.99 – 2.00) that was mostly harmful.

Periodontitis as an outcome

Table 7 shows the different associations of diabetes with the periodontitis outcome when periodontitis was defined using different cut-off points or thresholds in adjusted multivariable logistic regressions. The odds ratio of having periodontitis for diabetics compared to non-diabetics were equally distributed on either side of the null. The definitions of periodontitis resulted in widths of the 95% intervals ranging from (1.4 – 2.9). Most of the 95% confidence interval were compatible with lower a bound around 0.6-0.8 and were compatible with positive values (i.e., > 1) and negative values (i.e., < 1) alike.

Results were similar to the periodontitis-diabetes association in terms of changing the clinical parameters, extent, and severity. Stratified analysis (Table 8) showed the same results of the periodontitis-diabetes stratified analysis except that participants with 1-15 teeth had a range (0.63 – 3.45) that were mostly positive while those with > 15 teeth had a narrower range (0.69 – 1.29) that were mostly negative.

Discussion

In periodontitis as an exposure, results showed that the effect of clinical severity was association dependent, but clinical parameters (measures) and extent did not did depend on the association. The direction of the odds ratio in stratified analysis among those with 1-15 teeth also

depended on the association. In periodontitis as an outcome, it mostly exhibited similar patterns except for the direction of estimates among those with 1-15 teeth (protective vs. harmful).

The choice of measurement affects the measure of association between periodontitis as an exposure and systemic disease. Defining periodontitis by either clinical attachment loss or probing pocket depth increases the sensitivity of the definition and decreases its specificity while requiring both has the opposite effect. The choice of measurement of periodontitis as an exposure is likely to result in non-differential misclassification that would bias the estimates towards the null on average. Since the estimates from using either CAL or PD were similar to when using CAL only, this may suggest that using CAL only may result in similar sensitivity and specificity to when using either CAL or PD. Also, using PD only may have close sensitivity and specificity to when using both CAL and PD because they resulted in similar estimates.

The range of odds ratio was wider for the periodontitis-cardiovascular disease association compared to periodontitis-diabetes suggesting that the impact of misclassification of periodontitis as an exposure due to case definitions was more extreme in the cardiovascular disease association. This also suggests that the influence of CAL on the odds ratio of periodontitis as an exposure when using either CAL or PD to define periodontitis may depend on the nature of the relationship with the outcome under study.

The misclassification of periodontitis as an exposure that resulted from increasing the cut-off point was likely to be non-differential. Presence of trend with severity depended on the outcome. A possible explanation for this could be that the sensitivity and specificity of the definition based on severity interact with the choice of measurement differently in different outcomes.

Presence of a trend with extent did not depend on the association. A possible explanation for why the estimates tended to be the closest to the null as the severity or extent became most stringent was that more true cases of periodontitis could be misclassified as non-cases and thus would bias the estimates towards the null.

Stratified analysis showed that the estimates were wider in range among participants who had 1 – 15 teeth compared to those with > 15. A possible explanation for the periodontitis definitions to affect individuals with 1- 15 teeth more, was due to that stratum having a larger difference of misclassification by diabetes/CVD groups compared to the >15 teeth strata (assuming periodontitis causes diabetes/CVD). This is a special form of selection bias called prevalent cohort bias.

This analysis assumed that all definitions of periodontitis had the same model. However, some definitions of periodontitis may have had different functional forms with systemic disease (i.e., assumptions of additivity/no interaction). Using the same dataset for the different definitions of periodontitis did not make each periodontitis definition-systemic disease association independent and thus cannot be interpreted as evidence of dose-response (86).

In the periodontitis-CVD association, the direction of the association between periodontitis and diabetes was $diabetes \rightarrow periodontitis$ (i.e., diabetes caused periodontitis and so according to figure 1, diabetes was a confounder and was included in the model for the periodontitis-CVD association). However, the direction of the periodontitis and diabetes association could be the other way around $periodontitis \rightarrow diabetes$ (i.e., diabetes would be on the causal pathway from periodontitis to cardiovascular disease). In other words, since diabetes would be a mediator, including it in the model would bias the estimates of periodontitis

towards the null (underestimation). Therefore, this may have affected the estimates of the different definitions of periodontitis on cardiovascular diseases.

There was a chance that the causal diagrams were wrong about the data generating process of the periodontitis-systemic disease association. This may change the measures of association of different definitions of periodontitis.

The limitations of this study that could affect the results of the misclassification of periodontitis on the associations include the lack of temporality ascertainment of the exposure-outcome associations due to the cross-sectional nature. Furthermore, the misclassification of diabetes and cardiovascular disease due to self-report or misclassification of confounders could change the results of the study. In addition, the selection bias that could result from complete case analysis may have changed the percent relative bias. Moreover, the number of teeth required to be eligible for this study was at least 1 permanent tooth. However, having 1-6 teeth may not make a person at risk for some of the definitions used in this study and so to fairly compare between the definitions, at least 6 teeth should have been the minimum requirement to be eligible.

Perhaps taking a closer look into other definitions of periodontitis such as the international classification workshop definition. It could be that classification of periodontitis may be association dependent and that search a for universal definition may not be well thought out.

Conclusion

The effects of clinical severity on the odds ratios were association dependent. Clinical measures and extent did not depend on the association. The direction of the odds ratios among 1-

15 depended on the association. Interpretation of these results was limited by the lack of a gold standard (with respect to the type of bias).

Figures and Tables

Definition	Number of teeth	CAL in mm only	PD in mm only	CAL or PD in mm	CAL and PD in mm
A	1	3	3	3	3
B	1	4	4	4	4
C	1	5	5	5	5
D	1	6	6	6	6
E	2	3	3	3	3
F	2	4	4	4	4
G	2	5	5	5	5
H	2	6	6	6	6
I	3	3	3	3	3
J	3	4	4	4	4
K	3	5	5	5	5
L	3	6	6	6	6
M	4	3	3	3	3
N	4	4	4	4	4
O	4	5	5	5	5
P	4	6	6	6	6
Q	5	3	3	3	3
R	5	4	4	4	4
S	5	5	5	5	5
T	5	6	6	6	6

U	6	3	3	3	3
V	6	4	4	4	4
W	6	5	5	5	5
X	6	6	6	6	6

Table 1. Different definitions of periodontitis based on the number of teeth meeting millimeters of clinical attachment loss, probing pocket depth, clinical attachment loss or probing pocket depth, and both clinical attachment loss and probing pocket depth.

Variable	Frequency (weighted %)
Mean age (SE)	49.71 (0.22)
Missing	0 (0%)
Gender	
Male	5,001 (49.23%)
Missing	0 (0%)
Race/ethnicity	
Non-Hispanic White	4,161 (67.62%)
Non-Hispanic Black	2,161 (10.88%)
Mexican American and other Hispanic	2,547 (13.91%)
Other race and multiracial	1,278 (7.58%)
Missing	0 (0%)
Mean pack-years (SE)	7.42 (0.31)
Missing	242 (2.23%)
Mean waist/height ratio (SE)	0.59 (0.002)
Missing	315 (2.65%)
Mean number of teeth or root fragments (SE)	24.52 (0.10)
Mean number of teeth or root fragments measured (SE)	22.98 (0.12)
Diabetes	
Yes	1,239 (9.38%)
Missing	6 (0.03%)
Cardiovascular disease	

Yes	656 (5.57%)
Missing	32 (0.18%)
Total	10,147 (100%)

SE: Standard error.

Table 2. Characteristics of participants in NHANES cycles 2009-2014 aged 30-79 years, who had at least 1 permanent tooth during examination, and had complete periodontal examination (N=10,147).

Cut-off point	Number of teeth	CAL only		PD only		CAL or PD		CAL and PD*	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
3 mm	1	1.28	0.86–1.92	1.28	0.99-1.64	1.26	0.78-2.05	1.30	1.05-1.61
4 mm	1	1.08	0.89-1.31	1.05	0.85-1.30	1.10	0.91-1.33	1.04	0.83-1.32
5 mm	1	1.22	1.03-1.45	1.06	0.84-1.34	1.22	1.03-1.44	1.07	0.83-1.38
6 mm	1	1.08	0.87-1.36	0.99	0.74-1.33	1.09	0.88-1.36	0.97	0.71-1.32
3 mm	2	1.16	0.82-1.64	1.11	0.88-1.40	1.18	0.81-1.72	1.11	0.89-1.39
4 mm	2	1.24	1.02-1.50	1.08	0.87-1.33	1.24	1.04-1.49	1.07	0.85-1.36
5 mm	2	1.19	0.96-1.48	0.97	0.72-1.30	1.22	0.99-1.49	0.92	0.66-1.28
6 mm	2	1.08	0.84-1.40	0.93	0.62-1.39	1.04	0.82-1.33	1.01	0.67-1.54
3 mm	3	1.22	0.89-1.65	1.10	0.86-1.41	1.19	0.88-1.62	1.13	0.89-1.45
4 mm	3	1.12	0.92-1.36	1.00	0.79-1.26	1.14	0.94-1.38	0.97	0.75-1.27
5 mm	3	1.06	0.85-1.33	1.02	0.72-1.45	1.06	0.86-1.32	1.02	0.70-1.50
6 mm	3	0.97	0.77-1.23	0.87	0.57-1.33	0.93	0.74-1.17	0.98	0.62-1.53
3 mm	4	1.16	0.90-1.50	1.08	0.88-1.34	1.11	0.88-1.41	1.13	0.90-1.42
4 mm	4	1.14	0.93-1.41	0.97	0.75-1.25	1.16	0.94-1.41	0.94	0.71-1.26

5 mm	4	1.04	0.81-1.34	1.17	0.78-1.73	1.05	0.83-1.34	1.15	0.73-1.80
6 mm	4	1.07	0.83-1.36	1.05	0.67-1.62	1.01	0.79-1.28	1.26	0.79-2.00
3 mm	5	1.15	0.92-1.43	1.09	0.90-1.31	1.14	0.93-1.40	1.10	0.90-1.35
4 mm	5	1.19	0.96-1.47	0.97	0.72-1.31	1.20	0.97-1.49	0.94	0.68-1.30
5 mm	5	1.07	0.80-1.43	1.24	0.77-2.01	1.07	0.81-1.41	1.29	0.74-2.23
6 mm	5	0.98	0.75-1.28	0.87	0.59-1.29	0.94	0.73-1.21	1.02	0.68-1.54
3 mm	6	1.14	0.91-1.43	1.06	0.87-1.31	1.09	0.90-1.33	1.12	0.89-1.41
4 mm	6	1.15	0.92-1.44	1.10	0.82-1.49	1.20	0.95-1.51	1.03	0.74-1.43
5 mm	6	1.01	0.72-1.41	1.28	0.80-2.05	0.99	0.72-1.36	1.40	0.82-2.38
6 mm	6	0.90	0.65-1.24	0.99	0.65-1.52	0.90	0.66-1.22	1.03	0.71-1.50

***** Can be on the same tooth.

OR: odds ratio, **CI:** Confidence interval.

Table 3. Odds ratios of different thresholds of periodontitis in multivariable binary logistic regression with diabetes as an outcome and controlled for age, waist to height ratio, and pack-years in complete case analysis (N=9.593).

Cut-off point	Number of teeth	CAL only		PD only		CAL or PD		CAL and PD*	
		1-15	>15	1-15	>15	1-15	>15	1-15	>15
3 mm	1	2.06	1.21	1.12	1.32	2.02	1.18	1.16	1.32
4 mm	1	2.52	0.97	1.08	1.05	2.72	0.99	1.08	1.03
5 mm	1	1.48	1.16	0.86	1.09	1.47	1.16	0.86	1.10
6 mm	1	1.07	1.05	0.69	1.04	1.05	1.07	0.72	1.01
3 mm	2	1.44	1.11	1.31	1.08	1.29	1.15	1.39	1.07
4 mm	2	1.76	1.15	1.01	1.08	1.79	1.16	1.01	1.08
5 mm	2	1.01	1.19	0.75	1.00	0.99	1.23	0.77	0.94
6 mm	2	1.17	1.02	0.81	0.94	1.16	0.98	0.84	1.03
3 mm	3	1.66	1.14	1.24	1.08	1.45	1.14	1.37	1.10
4 mm	3	1.14	1.09	0.97	1.00	1.14	1.12	0.97	0.97
5 mm	3	0.99	1.05	0.74	1.07	0.98	1.05	0.77	1.07
6 mm	3	0.98	0.93	1.06	0.82	0.98	0.89	1.08	0.93
3 mm	4	1.76	1.08	1.60	1.03	1.83	1.02	1.57	1.08
4 mm	4	1.06	1.13	0.72	1.01	1.01	1.16	0.77	0.97

5 mm	4	0.91	1.04	0.81	1.22	0.91	1.06	0.82	1.20
6 mm	4	1.14	1.00	1.33	0.98	1.11	0.93	1.48	1.19
3 mm	5	1.65	1.07	1.41	1.06	1.78	1.05	1.32	1.08
4 mm	5	1.19	1.16	0.70	1.01	1.20	1.18	0.67	0.98
5 mm	5	0.95	1.07	0.93	1.28	0.94	1.07	0.94	1.34
6 mm	5	1.14	0.90	2.33	0.74	1.11	0.86	3.11	0.85
3 mm	6	1.28	1.12	1.35	1.04	1.31	1.06	1.34	1.10
4 mm	6	1.10	1.13	0.71	1.17	1.11	1.19	0.68	1.08
5 mm	6	0.94	1.00	1.78	1.24	0.94	0.98	1.81	1.36
6 mm	6	1.00	0.83	3.41	0.78	1.07	0.81	3.61	0.86

***** Can be on the same tooth.

Table 4. Odds ratios of different thresholds of periodontitis in multivariable binary logistic regression with diabetes as an outcome and controlled for age, waist to height ratio, and pack-years in complete case analysis stratified by the number of teeth 1-15 (N=967) vs.> 15 (N=8,626).

Cut-off point	Number of teeth	CAL only		PD only		CAL or PD		CAL and PD*	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
3 mm	1	1.27	0.87-1.85	1.03	0.80-1.32	1.16	0.77-1.74	1.10	0.86-1.39
4 mm	1	1.15	0.95-1.40	1.13	0.91-1.42	1.12	0.92-1.36	1.17	0.93-1.47
5 mm	1	1.15	0.91-1.45	1.08	0.80-1.46	1.12	0.89-1.40	1.13	0.83-1.53
6 mm	1	1.28	0.99-1.64	1.03	0.72-1.47	1.28	1.00-1.64	1.02	0.70-1.47
3 mm	2	1.12	0.86-1.46	1.03	0.78-1.34	0.91	0.67-1.24	1.16	0.91-1.48
4 mm	2	1.36	1.10-1.70	1.13	0.87-1.47	1.33	1.07-1.66	1.18	0.89-1.56
5 mm	2	1.36	1.06-1.74	1.27	0.89-1.82	1.28	1.00-1.64	1.42	1.00-2.04
6 mm	2	1.21	0.89-1.64	1.27	0.84-1.91	1.25	0.94-1.68	1.16	0.73-1.82
3 mm	3	1.20	0.96-1.50	1.08	0.85-1.37	1.09	0.83-1.43	1.16	0.93-1.44
4 mm	3	1.31	1.04-1.64	1.17	0.87-1.57	1.28	1.02-1.60	1.22	0.90-1.64
5 mm	3	1.40	1.04-1.88	1.51	0.97-2.34	1.34	0.99-1.81	1.67	1.09-2.57
6 mm	3	1.24	0.87-1.78	1.08	0.70-1.68	1.32	0.94-1.85	0.85	0.45-1.63
3 mm	4	1.24	0.98-1.56	1.07	0.84-1.35	1.16	0.92-1.46	1.14	0.90-1.44
4 mm	4	1.36	1.08-1.71	1.28	0.96-1.70	1.33	1.06-1.67	1.33	0.98-1.83

5 mm	4	1.36	0.98-1.88	1.66	1.07-2.57	1.37	0.99-1.89	1.68	1.05-2.71
6 mm	4	1.19	0.83-1.69	1.15	0.68-1.94	1.25	0.89-1.76	0.94	0.46-1.90
3 mm	5	1.22	1.00-1.50	1.22	0.96-1.55	1.16	0.93-1.44	1.30	1.01-1.67
4 mm	5	1.48	1.14-1.91	1.45	1.06-2.00	1.45	1.14-1.85	1.52	1.07-2.16
5 mm	5	1.25	0.85-1.84	1.62	1.04-2.52	1.32	0.92-1.90	1.49	0.89-2.51
6 mm	5	1.18	0.82-1.70	1.52	0.76-3.04	1.27	0.90-1.79	1.14	0.42-3.07
3 mm	6	1.36	1.08-1.70	1.20	0.94-1.53	1.26	0.99-1.60	1.31	1.03-1.67
4 mm	6	1.50	1.14-1.97	1.34	0.92-1.94	1.43	1.09-1.87	1.47	1.00-2.18
5 mm	6	1.34	0.87-2.05	1.63	1.00-2.65	1.46	0.98-2.18	1.32	0.73-2.41
6 mm	6	1.62	1.12-2.33	1.45	0.60-3.53	1.59	1.11-2.28	1.59	0.60-4.16

***** Can be on the same tooth.

OR: odds ratio, **CI:** Confidence interval.

Table 5. Odds ratios of different thresholds of periodontitis in multivariable binary logistic regression with cardiovascular disease as an outcome and controlled for age, waist to height ratio, and pack-years in complete case analysis (N=9.563).

Cut-off point	Number of teeth	CAL only		PD only		CAL or PD		CAL and PD*	
		1-15	>15	1-15	>15	1-15	>15	1-15	>15
3 mm	1	1.29	1.19	0.92	1.07	1.29	1.08	0.93	1.13
4 mm	1	0.78	1.12	0.69	1.23	0.71	1.10	0.72	1.26
5 mm	1	0.46	1.25	0.68	1.14	0.46	1.21	0.68	1.20
6 mm	1	0.69	1.35	0.92	1.03	0.68	1.36	0.94	1.00
3 mm	2	0.59	1.20	0.83	1.10	0.50	0.99	0.89	1.24
4 mm	2	0.62	1.45	0.54	1.27	0.58	1.43	0.57	1.31
5 mm	2	0.51	1.55	0.78	1.37	0.50	1.44	0.78	1.57
6 mm	2	0.68	1.27	0.79	1.38	0.67	1.35	0.83	1.23
3 mm	3	0.61	1.34	0.65	1.24	0.58	1.25	0.67	1.32
4 mm	3	0.58	1.45	0.62	1.31	0.54	1.44	0.70	1.35
5 mm	3	0.64	1.56	1.00	1.61	0.63	1.48	1.01	1.81
6 mm	3	0.74	1.30	0.27	1.35	0.73	1.41	0.28	1.06
3 mm	4	0.67	1.40	0.84	1.16	0.68	1.32	0.82	1.24
4 mm	4	0.66	1.50	0.84	1.36	0.66	1.47	0.85	1.43

5 mm	4	0.76	1.45	0.74	1.88	0.76	1.47	0.75	1.93
6 mm	4	0.81	1.16	0.32	1.42	0.80	1.26	0.33	1.15
3 mm	5	0.79	1.33	1.17	1.28	0.78	1.28	1.18	1.35
4 mm	5	0.71	1.64	0.89	1.59	0.69	1.63	0.94	1.65
5 mm	5	0.83	1.27	0.26	2.00	0.83	1.37	0.26	1.87
6 mm	5	0.92	1.08	0.38	1.88	0.91	1.21	0.40	1.41
3 mm	6	1.02	1.46	0.77	1.36	0.98	1.37	0.80	1.48
4 mm	6	0.74	1.67	0.87	1.44	0.74	1.58	0.87	1.60
5 mm	6	1.04	1.29	0.42	1.95	1.04	1.47	0.43	1.58
6 mm	6	1.38	1.42	0.24	1.89	1.29	1.45	0.50	1.87

***** Can be on the same tooth.

Table 6. Odds ratios of different thresholds of periodontitis in multivariable binary logistic regression with cardiovascular disease as an outcome and controlled for age, waist to height ratio, and pack-years in complete case analysis stratified by the number of teeth 1-15 (N=957) vs.> 15 (N=8,606).

Cut-off point	Number of teeth	CAL only		PD only		CAL or PD		CAL and PD*	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
3 mm	1	1.24	0.83-1.86	1.12	0.87-1.43	1.17	0.72-1.92	1.17	0.94-1.46
4 mm	1	1.02	0.83-1.25	0.97	0.78-1.20	1.04	0.85-1.26	0.96	0.75-1.21
5 mm	1	1.17	0.97-1.40	0.95	0.75-1.20	1.16	0.97-1.38	0.96	0.74-1.25
6 mm	1	1.02	0.82-1.28	0.92	0.68-1.23	1.03	0.83-1.29	0.89	0.65-1.22
3 mm	2	1.08	0.76-1.54	0.98	0.78-1.22	1.08	0.74-1.57	1.00	0.81-1.24
4 mm	2	1.18	0.96-1.45	0.97	0.78-1.20	1.18	0.97-1.43	0.97	0.76-1.23
5 mm	2	1.12	0.90-1.40	0.88	0.64-1.20	1.14	0.92-1.42	0.84	0.60-1.18
6 mm	2	0.99	0.76-1.29	0.87	0.58-1.31	0.95	0.74-1.22	0.95	0.62-1.45
3 mm	3	1.12	0.82-1.52	0.97	0.76-1.23	1.09	0.80-1.48	1.00	0.79-1.27
4 mm	3	1.05	0.86-1.28	0.89	0.70-1.15	1.06	0.87-1.29	0.87	0.66-1.16
5 mm	3	0.96	0.76-1.22	0.95	0.66-1.35	0.96	0.76-1.22	0.94	0.64-1.39
6 mm	3	0.90	0.71-1.14	0.80	0.52-1.23	0.86	0.68-1.08	0.90	0.58-1.39
3 mm	4	1.05	0.82-1.37	0.96	0.78-1.19	1.00	0.78-1.27	1.01	0.81-1.27
4 mm	4	1.07	0.86-1.32	0.87	0.66-1.14	1.07	0.87-1.32	0.85	0.62-1.16

5 mm	4	0.93	0.71-1.22	1.09	0.73-1.62	0.95	0.73-1.23	1.07	0.69-1.68
6 mm	4	0.98	0.76-1.26	0.95	0.61-1.48	0.92	0.72-1.18	1.15	0.72-1.82
3 mm	5	1.04	0.83-1.30	0.97	0.80-1.16	1.02	0.83-1.25	0.98	0.81-1.20
4 mm	5	1.10	0.88-1.36	0.88	0.64-1.19	1.10	0.88-1.37	0.85	0.61-1.21
5 mm	5	0.98	0.73-1.32	1.14	0.71-1.83	0.97	0.73-1.29	1.21	0.71-2.05
6 mm	5	0.91	0.69-1.20	0.81	0.55-1.20	0.87	0.67-1.14	0.95	0.63-1.44
3 mm	6	1.03	0.82-1.31	0.95	0.78-1.17	0.98	0.80-1.20	1.01	0.80-1.26
4 mm	6	1.06	0.84-1.33	1.01	0.74-1.37	1.10	0.87-1.39	0.94	0.67-1.32
5 mm	6	0.93	0.66-1.30	1.17	0.73-1.87	0.90	0.65-1.25	1.30	0.78-2.17
6 mm	6	0.83	0.59-1.15	0.93	0.60-1.45	0.83	0.60-1.14	0.94	0.65-1.35

***** Can be on the same tooth.

OR: odds ratio, **CI:** Confidence interval.

Table 7. Odds ratios of diabetes in multivariable binary logistic regression with periodontitis as an outcome defined using different criteria and controlled for age, waist to height ratio, and pack-years in complete case analysis (N=9.593).

Cut-off point	Number of teeth	CAL only		PD only		CAL or PD		CAL and PD*	
		1-15	>15	1-15	>15	1-15	>15	1-15	>15
3 mm	1	2.28	1.20	1.14	1.19	2.20	1.13	1.19	1.24
4 mm	1	2.74	0.91	1.08	0.97	2.99	0.93	1.07	0.96
5 mm	1	1.56	1.10	0.78	0.99	1.55	1.09	0.79	1.01
6 mm	1	1.11	0.98	0.65	0.97	1.09	1.00	0.67	0.94
3 mm	2	1.50	1.06	1.31	0.98	1.33	1.08	1.40	0.98
4 mm	2	1.90	1.09	0.98	0.99	1.94	1.09	0.97	0.99
5 mm	2	1.04	1.11	0.72	0.91	1.02	1.15	0.75	0.86
6 mm	2	1.17	0.93	0.74	0.88	1.15	0.89	0.76	0.97
3 mm	3	1.64	1.08	1.21	0.98	1.43	1.07	1.34	1.00
4 mm	3	1.14	1.02	0.91	0.91	1.13	1.04	0.92	0.88
5 mm	3	1.02	0.94	0.71	1.00	1.00	0.94	0.74	0.99
6 mm	3	1.02	0.87	0.95	0.77	1.01	0.82	0.96	0.87
3 mm	4	1.72	1.00	1.54	0.94	1.80	0.94	1.51	0.99
4 mm	4	1.09	1.06	0.67	0.92	1.03	1.08	0.73	0.88

5 mm	4	0.92	0.92	0.76	1.15	0.92	0.94	0.76	1.13
6 mm	4	1.19	0.92	1.18	0.91	1.16	0.86	1.32	1.11
3 mm	5	1.63	0.98	1.34	0.96	1.77	0.96	1.26	0.98
4 mm	5	1.26	1.07	0.66	0.93	1.26	1.08	0.63	0.90
5 mm	5	0.99	0.98	0.85	1.21	0.99	0.97	0.85	1.29
6 mm	5	1.18	0.86	2.26	0.69	1.15	0.82	3.04	0.80
3 mm	6	1.31	1.03	1.31	0.95	1.33	0.98	1.29	1.00
4 mm	6	1.15	1.04	0.67	1.08	1.15	1.10	0.64	1.00
5 mm	6	0.97	0.92	1.69	1.16	0.96	0.90	1.70	1.28
6 mm	6	1.00	0.79	3.45	0.74	1.07	0.78	3.40	0.81

***** Can be on the same tooth.

Table 8. Odds ratios of different thresholds of periodontitis in multivariable binary logistic regression with periodontitis as an outcome and controlled for age, waist to height ratio, and pack-years in complete case analysis stratified by the number of teeth 1-15 (N=967) vs.> 15 (N=8,626).

Chapter 3: Study 2

The Impact of Periodontitis Misclassification as an Exposure and as an Outcome from Partial-Mouth Measurements on its Association with Diabetes and Cardiovascular Disease Using NHANES

Introduction

The potential for bias due to partial-mouth measurement when assessing the effects of periodontitis' risk factors was recently evaluated empirically and in a simulation study (87, 88). The empirical study concluded that partial-mouth measurements may result in bias of the measure of association, and the magnitude and direction depend on the clinical severity used to define periodontitis. The authors of that study assessed the effects of periodontitis outcome misclassification resulting from different partial-mouth measurements using the CDC/AAP case definitions of periodontitis only. Thus, the effect of using different case definitions alone (i.e., other than CDC/AAP) with only full-mouth measurements on the associations between smoking, diabetes, alcohol, or obesity and (periodontitis) were not done (87). The simulation study only assessed one partial-mouth protocol (the random half-mouth). Effect of misclassification of partial-mouth measurements has not been widely assessed.

The mechanism of periodontitis misclassification depends on whether it is ascertained as an exposure or an outcome (49). Therefore, the potential for bias due to partial-mouth measurement may differ based on whether periodontitis is exposure or an outcome. The impact of potential bias resulting from partial-mouth with periodontitis as the exposure was not evaluated.

The aim of this paper was to study the impact of periodontitis misclassification resulting from use of several partial mouth protocols (PMPs) on its association as an exposure with

diabetes and cardiovascular disease and as an outcome with diabetes in relation to full-mouth measurements using the National Health and Nutrition Examination Survey (NHANES) 2009-2014.

Methods

We used data from NHANES 2009-2014. The 2009-2014 cycles were the only cycles with full-mouth measurements of CAL and PD on all six sites of each tooth. Thus, it allowed comparisons of several partial-mouth measurements to full-mouth measurement. Numbers of teeth present were calculated from permanent teeth and any permanent root fragments. Periodontitis was defined as a categorical variable according to the CDC/AAP case definition (67). Because of the low frequency of mild periodontitis, it was combined with the no periodontitis category Page and Eke 2007 periodontitis definition (67) using measurement on four interproximal sites per tooth (mesio-buccal, mesio-lingual, disto-buccal, and disto-lingual) for all 28 teeth (third molar excluded because there were no periodontal measurements for them in NHANES) (80, 81). A second definition, the mean clinical attachment loss, was based on the continuous CAL measurements on up to four interproximal sites per tooth.

Measurements of CAL and PD at four interproximal sites per tooth were used for the analysis periodontitis with full-mouth and each partial-mouth protocol. Fixed and random selection partial-mouth protocols are described below:

Fixed selection

In each of the following fixed selection protocols, teeth were selected one time. The protocols were the Community Periodontal Index of Treatment Needs (CPITN) index teeth (maxillary right first and second molars, maxillary right central incisor, maxillary left first and second molars, mandibular left first and second molars, mandibular left central incisor, and

mandibular right first and second molars) and the Ramfjörd teeth (maxillary right first molar, maxillary left central incisor, maxillary left first premolar, mandibular left first molar, mandibular right central incisor, and mandibular right first premolar). Additional six protocols were considered (appendix).

Random selection

Random selection occurred 10,000 times to account for random error of the sampling. A random number was generated in SAS for each subject using the binomial function with a probability of success of 0.5. The random half-mouth with contralateral sides (Mx Rt & Mn Lt / Mx Lt & Mn Rt) was used in which the maxillary right/mandibular left sides were selected if the Binomial value was 1, and the maxillary left/mandibular right sides if the value was 0. Additional two protocols were considered (appendix).

Statistical analysis

Multiple independent analyses were run to evaluate the role of periodontitis classification in periodontitis disease associations when periodontitis was the exposure of interest, i.e. exposure misclassification, and when periodontitis was the outcome of interest, i.e. outcome misclassification. In all cases, misclassification was assumed to be non-differential. All statistical analysis was carried out using SAS 9.4. Complete case analysis was conducted.

To evaluate exposure misclassification, Multivariable binary logistic regression models using were separately constructed for each outcome, i.e. diabetes and cardiovascular disease, with periodontitis as both a categorical and continuous (mean CAL) variable, were conducted for both full and partial-mouth protocols. Similarly, evaluations of outcome misclassification were conducted by constructing 1) multivariable generalized logistic (multinomial) models were done with diabetes as an exposure, periodontitis as an outcome 2) multivariable linear regression

models with diabetes as an exposure and mean clinical attachment loss as an outcome. These models were done using both full and partial-mouth protocols.

For all models, odds ratios or β-estimates and 95% CI were reported. Full-mouth estimate was subtracted from the corresponding partial-mouth estimate and then divided by the full-mouth models' estimate multiplied by a 100 to obtain the percent relative bias. For random partial-mouth techniques, the mean and (2.5^{th} - 97.5^{th}) percentile interval of percent relative bias were reported.

The regression models were conducted using proc survey options in SAS, taking into account the complex design of NHANES and exam weight. All models were adjusted for potential confounders. Figures 1 and 2 show the hypothesized causal diagrams of the periodontitis-diabetes and periodontitis-CVD associations, respectively. The minimally sufficient set of variables to control for in the periodontitis-diabetes/diabetes-periodontitis association based on the causal diagram below was age, gender, smoking, obesity, and race/ethnicity. For the periodontitis-CVD association, the minimally sufficient set of variables was age, gender, smoking, obesity, race/ethnicity, and diabetes. No model selection procedures were conducted.

Stratified and sensitivity analysis

To assess the potential impact of varying numbers of teeth across participants, we estimated associations among those with less than or equal to 15 and among those with more than 15 teeth. In addition, reanalysis with different eligibility criteria of subjects with at least 8, 10, 20, and full 28 teeth (excluding 3^{rd} molars) present was done to investigate the potential effects of losing teeth on the measures of association. These stratified/sensitivity analysis attempt to identify the potential impact of prevalent cohort bias, a special form of left truncation or selection bias, in which subjects who already had periodontitis and lost their teeth due to

periodontitis may either not make it into the analytic cohort, or may be misclassified as a non-case of periodontitis (85).

Results

The sample eligible for this analysis was 10,138. The full eligible sample had a mean age of 49.70 (with a standard error of 0.22), was 49.20% males, and the majority had non-Hispanic white (67.62%). Diabetes was in 9.35% and cardiovascular disease was in 5.54% of the sample. The prevalence of severe, moderate, mild, and no periodontitis were 7.80%, 29.00%, 4.42%, and 58.77%, respectively. The sample had missing diabetes status, cardiovascular disease status, pack-years, and waist to height ratio that were 0.03%, and 0.18%, 2.23%, 2.65%, respectively. Tables 9 shows the distribution of the variables by periodontitis and diabetes groups.

Full-mouth adjusted analysis are shown in Table 10. When periodontitis was an exposure and CDC/AAP definition was used, those with severe periodontitis had 1.09 (95% CI: 0.77, 1.54) times the odds of having diabetes compared to those with mild or no periodontitis. Moderate periodontitis had 1.30 (95% CI: 1.07, 1.57) times the odds of having diabetes compared to those with mild or no periodontitis. For mean CAL, each 1 mm increase in clinical attachment loss was associated with a 6% increase in the odds (OR=1.06) of having diabetes (95% CI: 0.98, 1.14). Furthermore, each 1 mm increase in clinical attachment loss was associated with a 14% increase in the odds (OR=1.14) of having cardiovascular disease (95% CI: 1.06, 1.22).

In periodontitis as CDC/AAP outcome, diabetics had 1.01 times the odds of having severe periodontitis (as opposed to mild or no periodontitis) compared to non-diabetics with a compatibility interval ranging from 0.72-1.43. Diabetics had 1.25 times the odds of having moderate periodontitis (as opposed to mild or no periodontitis) compared to non-diabetics with a

compatibility interval ranging from 1.02-1.52. For mean CAL, diabetics had 0.05 higher mean of clinical attachment loss compared to non-diabetics with a compatibility interval ranging from -0.04 to 0.14.

Exposure misclassification

Categorical Periodontitis

When periodontitis was evaluated as a categorical exposure, partial-mouth protocols mainly resulted in underestimation (Table 11). On average, the percent relative bias was less than 10% for all protocols and both associations (periodontitis-diabetes and periodontitis-CVD). However, the effects of moderate periodontitis on diabetes/CVD had more bias than the effects of severe periodontitis. Those with severe periodontitis had 1.02, 1.16, and 1.14 times the odds of having diabetes and 1.35, 1.22, and 1.38 times of having CVD compared to those with mild/no periodontitis using the random half-mouth, CPITN, and the Ramfjörd protocols, respectively (Table 12). Those with moderate periodontitis had 1.19, 1.17, and 1.14 times the odds of having diabetes and 1.33, 1.38, and 1.18 times of having CVD compared to those with mild/no periodontitis using the random half-mouth, CPITN, and the Ramfjörd protocols, respectively.

When comparing the percent relative bias of the effects of severe periodontitis on diabetes/CVD across the different partial-mouth protocols, there was no consistent bias in terms of size and direction as the partial-mouth protocol had fewer teeth. However, as the partial-mouth protocol had fewer teeth, the bias was increased when examining the effects of moderate periodontitis on diabetes/CVD (Table 11).

Continuous Periodontitis (mean CAL)

When periodontitis was evaluated as a continuous exposure using mean CAL, the partial-mouth protocols resulted in minimal bias overall compared to when periodontitis was ascertained categorically (Table 11).

Outcome misclassification

Categorical Periodontitis

The effect of diabetes on severe periodontitis consistently resulted in less than 10% bias while the effects of diabetes on severe periodontitis consistently exceeded 10% bias (Table 13). Comparisons across PMPs showed similar results to when periodontitis was an exposure (i.e., no trend with effects of diabetes on severe periodontitis but clear trend with effects on moderate periodontitis). Diabetes had 0.94, 1.04, and 1.08 times the odds of having severe periodontitis compared to non-diabetics using the random half-mouth, CPITN, and the Ramfjörd protocols, respectively (Table 14). Diabetics had 1.11, 1.07, and 1.07 times the odds of having diabetes compared to non-diabetics using the random half-mouth, CPITN, and the Ramfjörd protocols, respectively.

Continuous Periodontitis (mean CAL)

As a continuous variable, mean CAL resulted in higher percent relative bias compared to the CDC/AAP definition (Table 13).

Stratified and sensitivity analysis

When results were stratified by number of teeth (2-15 vs >15), percent relative bias was higher among those with 2-15 teeth compared to those with more than 15 teeth regardless whether periodontitis was an exposure or an outcome and regardless of the outcome for periodontitis (diabetes/CVD) (Tables 11 and 13). In periodontitis as an exposure, the magnitude and direction of bias among those with 2-15 teeth was association dependent (i.e., more bias in

the periodontitis-diabetes association). In addition, bias was minimal and in several cases not present when periodontitis was an exposure. Furthermore, the use of mean CAL resulted in the least amount of bias compared to CDC/AAP even after stratification.

The percent relative bias was mixed in direction and extent (i.e., no trend with more restriction) (Tables 11 and 13). However, restriction to 28 teeth generally resulted in the highest bias. Moreover, the Ramfjörd protocol tended to be the most affected by restriction to all 28 teeth.

Discussion

Periodontitis as a CDC/AAP exposure on average had <10% relative bias and mainly resulted in underestimation. The bias was larger for the effects of moderate periodontitis compared to the effects of severe periodontitis. As the partial-mouth protocol had less number of teeth, the bias was larger for the effects of moderate periodontitis while effects of severe periodontitis had no trend. Participants with fewer teeth had more bias than those with more teeth. The direction of bias depended on the outcome (diabetes vs. CVD) across strata of teeth. Mean CAL had minimal less bias compared to CDC/AAP definition.

Periodontitis as a CDC/AAP outcome generally had <10% relative bias when estimating effects of diabetes on severe periodontitis but had >10% when estimating its effects on moderate periodontitis. The direction of bias was mixed for severe but was to the null for moderate periodontitis. Trends among different partial-mouth protocols showed similar results as in when periodontitis was an exposure and so did the stratified analysis. Mean CAL had larger bias compared to CDC/AAP definition.

Periodontitis as an exposure had three categories and non-differential misclassification is expected to bias the severe category towards the null and bias the moderate category away from

the null since the severe and moderate category will look more similar (some severe move into moderate and vice-versa), i.e., if there was a trend or dose-response in the estimates. However, this was not the case in this study since there was no dose-response in both outcomes. Moderate periodontitis had more bias than severe periodontitis in diabetes. A possible explanation for this could be due to CDC definition capturing more moderate cases than severe cases and the higher estimate of the effect of moderate periodontitis. Thus, individuals with severe periodontitis were misclassified as either moderate or no periodontitis and individuals with moderate periodontitis were misclassified as no periodontitis. Therefore, the reference group (mild/none) was more similar to moderate periodontitis due to the misclassification and had more potential for bias. Thus, the true potential of bias in severe periodontitis may have been masked if the estimate of severe periodontitis is truly large.

Participants with fewer teeth were more impacted by partial-mouth misclassification than the ones with more teeth. Therefore, the number of teeth can have a major effect on bias from partial-mouth misclassification. A potential explanation for this could be that individuals who already had severe or moderate periodontitis lost their most affected teeth in different areas of the mouth so that when full-mouth measurements were done, they were classified as severe or moderate but when partial-mouth measurement were done, they were classified as moderate or mild/none. The direction of bias among participants with fewer teeth differed with the outcome and the reason for this remains to be unknown. This needs further investigation.

Restriction analysis to a higher number of teeth did not change the bias much except for restricting to all teeth. Mean clinical attachment loss was still affected by prevalent cohort bias but to a lesser extent for restriction to ≥8 or ≥20 teeth compared to restriction to all 28 teeth. However, it was affected to a lesser degree compared to the CDC/AAP categorical periodontitis.

Misclassification from partial-mouth measurements is expected to be non-differential. For periodontitis associations both as an exposure and as an outcome with diabetes, the sensitivity and specificity should be exactly the same yet, periodontitis as an outcome was more affected by misclassification from partial-mouth measurements than as an exposure. Another difference was that mean clinical attachment loss had more bias than the CDC/AAP definition. A possible reason for this was the already low beta-estimate of the full-mouth making any small change in estimate result in high percent relative bias. This may be due to the nature of exposure vs. outcome misclassification. Although stratified and restriction analysis had the same impact on periodontitis as an outcome.

The Ramfjörd protocol was among the highest percent relative bias for both periodontitis as an exposure and as an outcome when restricting the number of teeth. A potential reason could be that it only has six teeth of which two are only molars and four are only posterior teeth. Thus, it had the most bias because it left out the other most prone teeth to periodontitis.

A drawback of using percent relative bias is that it depends on the original value. A slight change to a low base value tends to result in a higher relative bias than if the base value is high even though the absolute change is the same (as in the case of b-estimate of diabetes on mean clinical attachment loss). A possible reason for this could be that the causal diagrams were wrong about the data generating process of the periodontitis-systemic disease association. This may change the estimates of severe categories and in turn, change the bias of the partial-mouth measurements. In addition, the cross-sectional nature of this analysis makes temporality of the associations questionable.

In the periodontitis-CVD association, the direction of the association between periodontitis and diabetes was $diabetes \rightarrow periodontitis$ (i.e., diabetes caused periodontitis

and so according to appendix figure 1, diabetes was a confounder was included in the model for the periodontitis-CVD association). However, the direction of the periodontitis and diabetes association could be the other way around $periodontitis \rightarrow diabetes$ (i.e., diabetes would be on the causal pathway from periodontitis to cardiovascular disease). In other words, since diabetes would be a mediator, including it in the model would bias the estimates of periodontitis towards the null (underestimation). Therefore, this may have affected the relative bias change percentages of different partial-mouth protocols for periodontitis-CVD association.

The assumptions of some of the models may have been violated and if changes were made to fix these assumptions, the relative bias change of the partial-mouth protocols may be affected. Furthermore, outliers and high influence points were not checked and may change the relative bias change percent of the partial-mouth techniques. Moreover, this analysis assumes that partial-mouth models follow full-mouth ones. However, partial-mouth models of the periodontitis-systemic disease associations may follow a different functional form of the models (assumptions of additivity/no interaction, linearity, normality, constant variance) from than the full-mouth one.

The multinomial logistic regression model was used instead of the proportional odds (cumulative) logistic regression model was due to violation of the proportional odds assumption that was assessed through significance of the score test and mosaic plots of the joint distribution of diabetes and periodontitis (89).

The limitations of this study that could affect the results of the misclassification of periodontitis on the associations include the lack of temporality ascertainment of the exposure-outcome associations due to the cross-sectional nature. Furthermore, the misclassification of diabetes and cardiovascular disease due to self-report or misclassification of confounders could

change the results of the study. Last, the selection bias that could result from complete case analysis may have changed the percent relative bias.

Conclusion

The amount of bias due to PMPs may be dependent on the size of the underlying association. The choice of protocol did not seem to play a major role in the amount of bias (number of teeth vs. type of tooth included). Percent relative bias was generally less than 10% for the severe categories while it tended to exceed 10% for moderate categories. Bias was increased among people with fewer teeth and the direction depended on the outcome. Mean clinical attachment loss resulted in minimal bias. Overall, bias due to PMP would not have changed the main conclusions when severe periodontitis was under study.

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Tables

Variables	Frequency (weighted %)					
	By periodontitis groups				By diabetic groups	
	Severe	**Moderate**	**Mild**	**None**	**Diabetic**	**Non-diabetic**
Mean age (SE)	53.63 (0.48)	53.69 (0.39)	46.87 (0.67)	47.41 (0.28)	57.43 (0.43)	48.90 (0.22)
Missing	0	0	0	0	0	0
Gender						
Male	814 (72.43%)	1,902 (55.90%)	265 (57.91%)	2,012 (42.15%)	635 (52.10%)	4,357 (48.92%)
Missing	0	0	0	0	0	0
Race/ethnicity						
Non-Hispanic White	300 (50.82%)	1,195 (59.70%)	187 (60.93%)	2,475 (74.26%)	375 (57.84%)	3,780 (68.64%)
Non-Hispanic Black	355 (20.36%)	850 (13.58%)	103 (13.71%)	851 (8.09%)	355 (16.12%)	1,804 (10.35%)
Mexican American and other Hispanic	346 (19.74%)	991 (17.96%)	157 (20.29%)	1,051 (10.66%)	366 (16.90%)	2,176 (13.59%)
Other race and multiracial	139 (9.08%)	428 (8.76%)	35 (5.07%)	675 (6.99%)	140 (9.13%)	1,136 (7.42%)
Missing	0	0	0	0	0	0

Mean pack-years						
(SE)	15.82 (1.06)	12.04 (0.52)	4.67 (0.60)	4.22 (0.23)	12.39 (1.12)	6.90 (0.30)
Missing	38 (2.09%)	82 (2.09%)	12 (2.18%)	110 (2.33%)	25 (1.77%)	217 (2.28%)
Mean waist to						
height ratio (SE)	0.60 (0.004)	0.61 (0.003)	0.60 (0.006)	0.58 (0.002)	0.66 (0.004)	0.59 (0.002)
Missing	41 (3.56%)	127 (3.35%)	7 (1.09%)	138 (2.30%)	62 (5.10%)	251 (2.39%)
Diabetes						
Yes	189 (13.31%)	593 (14.24%)	43 (8.85%)	411 (6.46%)	----------	----------
Missing	1 (0.04%)	2 (0.03%)	0	3 (0.03%)	----------	----------
Cardiovascular						
disease						
Yes	106 (8.84%)	330 (9.01%)	18 (3.17%)	199 (3.56%)	----------	----------
Missing	6 (0.36%)	19 (0.32%)	1 (0.09%)	6 (0.09%)	----------	----------

Periodontitis						
Severe	----------	----------	----------	----------	189 (11.10%)	950 (7.46%)
Moderate	----------	----------	----------	----------	593 (44.14%)	2,869 (27.44%)
Mild	----------	----------	----------	----------	43 (4.18%)	439 (4.45%)
None	----------	----------	----------	----------	411 (40.57%)	4,638 (60.65%)
Missing	----------	----------	----------	----------	0	0
Total	1,140 (7.80%)	3,464 (29.00%)	482 (4.42%)	5,052 (58.77%)	1,236 (9.35%)	8,896 (90.62%)

SE: Standard error.

***** 6 individuals were missing diabetes status (0.03% of the eligible sample).

Table 9. Characteristics of participants in NHANES cycles 2009-2014 aged 30-79 years, who had at least 2 permanent teeth during examination, one of which was not a root fragment, and had complete periodontal examination (N=10,138).

Variable	Categorical Perio								Mean CAL					
	Perio - Db*		Perio - CVD**		Db - Perio***				Perio - Db*		Perio - CVD**		Db - Perio****	
					Severe vs ref		Moderate vs ref							
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	β	95% CI
Perio														
Severe	1.09	(0.77-1.54)	1.36	(0.92-2.00)										
Moderate	1.30	(1.07-1.57)	1.37	(1.11-1.69)										
Mild/ none	Ref	-	Ref	-										
Mean CAL									1.06	(0.98-1.14)	1.14	(1.06-1.22)		
Diabetes														
Yes					1.01	(0.72-1.43)	1.25	(1.02-1.52)					0.05	(-0.04,0.14)
No					Ref	-	Ref	-						

Perio: periodontitis, **Db:** diabetes, **CVD:** cardiovascular disease, **OR:** odds ratio, **CI:** confidence interval, **β:** beta-estimate.
* Multivariable binary logistic regression adjusted for age (continuous), pack-years (continuous), and waist to height ratio (continuous).
** Multivariable binary logistic regression adjusted for age (continuous), pack-years (continuous), waist to height ratio (continuous), and diabetes (yes/no or borderline).
*** Multivariable multinomial logistic regression adjusted for age (continuous), pack-years (continuous), and waist to height ratio (continuous), **severe vs ref:** is when the outcome of severe periodontitis was compared to no or mild periodontitis, **moderate vs ref:** is when the outcome of moderate periodontitis was compared to no or mild periodontitis.
**** Multivariable linear regression adjusted for age (continuous), pack-years (continuous), and waist to height ratio (continuous).

Table 10. Full-mouth analysis of the periodontitis-diabetes and diabetes-periodontitis associations (N=9,586) and the periodontitis-cardiovascular disease (N=9,556) using both categorical definition and mean clinical attachment loss.

Partial-mouth protocol (number of teeth)	Exposure misclassification					
	Periodontitis - Diabetes			Periodontitis – Cardiovascular disease		
	Severe[ʊ]	Moderate[§]	Mean CAL	Severe[ʊ]	Moderate[§]	Mean CAL
Random half-mouth (14)*						
≥ 2 teeth	-6.42%	-8.46%	-0.94%	-0.74%	-2.19%	-0.88%
> 15 / ≤ 15 teeth	-4.72% / -27.07%	-2.52% / -44.50%	0.00% / -0.96%	-1.43% / 23.08%	1.37% / 3.13%	0.00% / 2.17%
≥ 8 teeth	-7.34%	-9.92%	-0.93%	0.74%	0.75%	0.00%
≥ 20 teeth	-9.17%	-3.28%	-0.94%	2.50%	-0.71%	-0.80%
28 teeth	22.22%	0.00%	0.00%	-20.00%	-12.97%	-0.82%
CPITN (10)						
≥ 2 teeth	6.42%	-10.00%	-0.94%	-10.29%	0.73%	-4.39%
> 15 / ≤ 15 teeth	10.38% / -14.29%	0.00% / -51.20%	0.00% / -1.92%	-5.00% / 29.23%	7.53% / 14.06%	-4.20% / 2.17%
≥ 8 teeth	3.67%	-12.21%	-2.78%	-8.15%	5.22%	-6.03%
≥ 20 teeth	4.59%	-6.56%	-1.89%	3.75%	7.80%	-4.00%
28 teeth	24.07%	1.49%	-1.25%	4.71%	15.14%	-4.10%

Ramfjörd (6)						
≥ 2 teeth	4.59%	-12.31%	-0.94%	1.47%	-13.87%	-1.75%
> 15 / ≤ 15 teeth	9.43% / -43.61%	-5.88% / -47.85%	1.90% / -5.77%	15.00% / -29.23%	-8.90% / -14.06%	-0.84% / 0.00%
≥ 8 teeth	3.67%	-12.98%	0.00%	2.96%	-8.96%	-1.72%
≥ 20 teeth	8.26%	-9.02%	2.83%	38.13%	-6.38%	-2.40%
28 teeth	-100.00%	34.33%	7.50%	-100.00%	5.41%	-3.28%

CPITN: community periodontal index for treatment needs.
ʊ: Percent relative bias of odds ratio of severe periodontitis on diabetes or cardiovascular disease compared to no or mild periodontitis.
§: Percent relative bias of odds ratio of moderate periodontitis on diabetes or cardiovascular disease compared to no or mild periodontitis.
***** Percent relative bias of the mean estimate of 10,000 iterations.

Table 11. Percent relative bias of the estimates of the different partial-mouth protocols for the periodontitis-diabetes (N=9,586) and the periodontitis-cardiovascular disease (N=9,556) associations using both categorical definition and mean clinical attachment loss compared to full-mouth estimates.

Full-mouth / partial-mouth protocol (number of teeth)	Exposure misclassification					
	Periodontitis - Diabetes			Periodontitis – Cardiovascular disease		
	Severe[ϖ]	Moderate[§]	Mean CAL	Severe[ϖ]	Moderate[§]	Mean CAL
Full-mouth (28)						
≥ 2 teeth	1.09	1.30	1.06	1.36	1.37	1.14
> 15 / ≤ 15 teeth	1.06 / 1.33	1.19 / 2.09	1.05 / 1.04	1.40 / 0.65	1.46 / 0.64	1.19 / 0.92
≥ 8 teeth	1.09	1.31	1.08	1.35	1.34	1.16
≥ 20 teeth	1.09	1.22	1.06	1.60	1.41	1.25
28 teeth	0.54	0.67	0.80	2.55	2.31	1.22
Random half-mouth (14)*						
≥ 2 teeth	1.02	1.19	1.05	1.35	1.34	1.13
	(0.88, 1.17)	(1.08, 1.30)	(1.04, 1.07)	(1.34, 1.58)	(1.20, 1.50)	(1.12, 1.15)
> 15 / ≤ 15 teeth	1.01 / 0.97	1.16 / 1.16	1.05 / 1.03	1.38 / 0.80	1.48 / 0.66	1.19 / 0.94
> 15 interval	(0.85, 1.19)	(1.05, 1.29)	(1.03, 1.07)	(1.12, 1.67)	(1.30, 1.67)	(1.17, 1.21)
≤ 15 interval	(0.75, 1.22)	(0.90, 1.46)	(1.00, 1.07)	(0.62, 1.02)	(0.52, 0.82)	(0.90, 0.98)

≥ 8 teeth	1.01	1.18	1.07	1.36	1.35	1.16
	(0.87. 1.16)	(1.07, 1.30)	(1.06, 1.09)	(1.14, 1.60)	(1.21, 1.51)	(1.14, 1.18)
≥ 20 teeth	0.99	1.18	1.05	1.64	1.40	1.24
	(0.79, 1.20)	(1.06, 1.31)	(1.03, 1.07)	(1.27, 2.05)	(1.22, 1.60)	(1.21, 1.27)
28 teeth	0.66	0.67	0.80	2.04	1.61	1.21
	(0.32, 1.10)	(0.53, 0.83)	(0.75, 0.85)	(0.63, 3.81)	(1.07, 2.25)	(1.11, 1.32)
CPITN (10)						
≥ 2 teeth	1.16	1.17	1.05	1.22	1.38	1.09
> 15 / ≤ 15 teeth	1.17 / 1.14	1.19 / 1.02	1.05 / 1.02	1.05 / 0.84	1.57 / 0.73	1.14 / 0.94
≥ 8 teeth	1.13	1.15	1.05	1.24	1.41	1.09
≥ 20 teeth	1.14	1.14	1.04	1.66	1.52	1.20
28 teeth	0.67	0.68	0.79	2.67	2.13	1.17

Ramfjörd (6)

≥ 2 teeth	1.14	1.14	1.05	1.38	1.18	1.12
> 15 / ≤ 15 teeth	1.16 / 0.75	1.12 / 1.09	1.07 / 0.98	1.61 / 0.46	1.33 / 0.55	1.18 / 0.92
≥ 8 teeth	1.13	1.14	1.08	1.39	1.22	1.14
≥ 20 teeth	1.18	1.11	1.09	2.21	1.32	1.22
28 teeth	0.000002	0.90	0.86	0.000002	1.95	1.18

CPITN: community periodontal index for treatment needs.
ʊ: Odds ratio of severe periodontitis on diabetes or cardiovascular disease compared to no or mild periodontitis.
§: Odds ratio of moderate periodontitis on diabetes or cardiovascular disease compared to no or mild periodontitis.
***** Mean estimates and (2.5^{th}, 97.5^{th}) percentile intervals of 10,000 iterations.

Table 12. Estimates of the different partial-mouth protocols for the periodontitis-diabetes (N=9,586) and the periodontitis-cardiovascular disease (N=9,556) associations using both categorical definition and mean clinical attachment loss.

Partial-mouth protocol (number of teeth)	Outcome misclassification		
	Severe[¥]	Moderate[‡]	Mean CAL*
Random half-mouth (14)**			
≥ 2 teeth	-6.93%	-11.20%	0.00%
> 15 / ≤ 15 teeth	-4.08% / -29.41%	-4.42% / -47.19%	0.00% / 6.67%
≥ 8 teeth	-7.92%	-12.00%	0.00%
≥ 20 teeth	-9.90%	-6.72%	0.00%
28 teeth	15.38%	3.33%	0.00%
CPITN (10)			
≥ 2 teeth	2.97%	-14.40%	-20.00%
> 15 / ≤ 15 teeth	9.18% / -20.59%	-2.65% / -55.41%	50.00% / -20.00%
≥ 8 teeth	0.99%	-15.20%	-16.67%
≥ 20 teeth	2.97%	-10.08%	0.00%
28 teeth	9.62%	1.67%	25.00%
Ramfjörd (6)			
≥ 2 teeth	6.93%	-14.40%	-20.00%
> 15 / ≤ 15 teeth	14.29% / -47.06%	-7.08% / -52.81%	100.00% / 140.00%
≥ 8 teeth	5.94%	-15.20%	0.00%

≥ 20 teeth	7.92%	-12.61%	100.00%
28 teeth	-100.00%	40.00%	-25.00%

CPITN: community periodontal index for treatment needs.
¥: Percent relative bias of odds ratio of diabetics for having severe periodontitis against no or mild periodontitis compared to non-diabetics.
‡: Percent relative bias of odds ratio of diabetics for having moderate periodontitis against no or mild periodontitis compared to non-diabetics.
***** Percent relative bias of beta-estimate of diabetics for mean clinical attachment loss compared to non-diabetics.
****** Percent relative bias of the mean estimate of 10,000 iterations.

Table 13. Percent relative bias of the estimates of the different partial-mouth protocols for the diabetes-periodontitis associations (N=9,586) using both categorical definition and mean clinical attachment loss compared to full-mouth estimates.

Full-mouth / partial-mouth protocol (number of teeth)	Outcome misclassification		
	Severe[¥]	Moderate[‡]	Mean CAL*
Full-mouth (28)			
≥ 2 teeth	1.01	1.25	0.05
> 15 / ≤ 15 teeth	0.98 / 1.36	1.13 / 2.31	0.02 / 0.15
≥ 8 teeth	1.01	1.25	0.06
≥ 20 teeth	1.01	1.19	0.02
28 teeth	0.52	0.60	-0.08
Random half-mouth (14)**			
≥ 2 teeth	0.94 (0.82, 1.08)	1.11 (1.00, 1.21)	0.05 (0.03, 0.07)
> 15 / ≤ 15 teeth	0.94 / 0.96 (0.79, 1.10) / (0.73, 1.22)	1.08 / 1.22 (0.98, 1.19) / (0.95, 1.54)	0.02 / 0.16 (0.01, 0.04) / (0.06, 0.26)
≥ 8 teeth	0.93 (0.80, 1.07)	1.10 (1.00, 1.21)	0.06 (0.04, 0.07)

≥ 20 teeth	0.91 (0.73, 1.10)	1.11 (0.99, 1.23)	0.02 (0.003, 0.03)
28 teeth	0.60 (0.32, 0.94)	0.62 (0.49, 0.76)	-0.08 (-0.09, -0.06)
CPITN (10)			
≥ 2 teeth	1.04	1.07	0.04
> 15 / ≤ 15 teeth	1.07 / 1.08	1.10 / 1.03	0.03 / 0.12
≥ 8 teeth	1.02	1.06	0.05
≥ 20 teeth	1.04	1.07	0.02
28 teeth	0.57	0.61	-0.10
Ramfjörd (6)			
≥ 2 teeth	1.08	1.07	0.04
> 15 / ≤ 15 teeth	1.12 / 0.72	1.05 / 1.09	0.04 / -0.06
≥ 8 teeth	1.07	1.06	0.06
≥ 20 teeth	1.09	1.04	0.04
28 teeth	0.000005	0.84	-0.06

CPITN: community periodontal index for treatment needs.

¥: Odds ratio of diabetics for having severe periodontitis against no or mild periodontitis compared to non-diabetics.
‡: Odds ratio of diabetics for having moderate periodontitis against no or mild periodontitis compared to non-diabetics.
***** Beta-estimate of diabetics for mean clinical attachment loss compared to non-diabetics.
****** Mean estimates and (2.5^{th}, 97.5^{th}) percentile intervals of 10,000 iterations.

Table 14. Estimates of the different partial-mouth protocols for the diabetes-periodontitis associations (N=9,586) using both categorical definition and mean clinical attachment loss.

Chapter 4: Study 3

The Impact of Unmeasured Confounding on Periodontitis' Associations with Diabetes and Cardiovascular Disease in the Dental Longitudinal Study (DLS)

Introduction

The observed associations, both their presence and/or strength, between periodontitis and diabetes and cardiovascular disease could also be explained by systematic errors including measurement/misclassification bias, selection bias, or confounding, including residual confounding or unmeasured confounding. Given the common pathways of periodontitis, diabetes, and CVD, the possibility exists that the observed associations reflect unmeasured confounding. Several techniques have been introduced in the epidemiology literature to address the impact of systematic errors, including unmeasured confounding. Bias analysis (a subset of sensitivity analysis) is one method that has been proposed (50, 90). The method is implemented by independently simulating the association between the hypothesized unmeasured confounder and the exposure, and the association with the outcome.

The plausible range of estimated odds ratios is extracted from the literature if the unmeasured confounder is known or these values can be simulated if there the unmeasured confounder is either not known or there nothing in the literature on that unmeasured confounder's association with periodontitis/diabetes. These values of odds ratios between the unmeasured confounder and periodontitis/diabetes are used to generate simulated datasets from which the odds ratio between the unmeasured confounder and periodontitis/diabetes in the dataset can be estimated.

This study aims to investigate the possible presence and potential effect of an unknown unmeasured confounder on the association between periodontitis and diabetes and cardiovascular disease using the Dental Longitudinal Study (DLS).

Methods

Data source

The Dental Longitudinal Study (DLS) is a subset of the Normative Aging Study (NAS) from the Department of Veterans Affairs (VA). The NAS is a cohort of male veterans who were living in Boston, MA in 1961. Study procedures were conducted at a VA outpatient clinic in the Boston area. Subjects were recruited through advertising in newspapers, radio, and soliciting police department and large insurance companies. The primary purposes of the study were to observe the natural process of aging in a healthy population, study incidence of disease accompanied by aging, and assess different lifestyle factors and how they affect aging. Veterans were selected as the target population as they were a representative sample of all adult males at the time the study was initiated given that85% of all eligible males of military age participated in World War II. In addition, their data could be gathered retrospectively though VA records and follow-up was possible. Another reason for choosing veterans was the national network of VA making it easier to follow-up even if they left the Boston area. Investigators stressed that only healthy individuals who did not have a major disease or injury should apply. Recruitment was initiated in 1961 and continued until 1970. Over 6,000 individuals were recruited and only 2,280 passed the strict criteria of inclusion. Participants were healthy men, who had varying socioeconomic status, occupation, mostly white (98%), mostly married and with children of school age and were willing to continue their jobs and intend to participate in the study in further cycles. All of this made the study population geographically stable. To maintain the population,

attempts of sending periodic newsletters and involvement of individuals in a network were undertaken. (91-94).

The DLS started in 1968 by asking individuals participating in the NAS to participate via self-selection. Self-selection could have been due to interest in dental health, the need of dental treatment, compliance with parent study, or peer pressure by siblings/coworkers. Dental status was not ascertained prior to enrollment into the DLS. The recruited NAS cohort was 2,280 participants in 1968 and only 1,231 agreed to participate in the DLS (62%). At baseline, ages ranged from 25-75. Data for this study included data obtained on participants through the year 2009. Figure 4 shows the timeline of the two cohorts and the follow-up rate, including the timing of diabetes and periodontitis measurements. The follow-up rate for the DLS was not high and drop out was highest in the first cycle losing 16% of the baseline cohort (95, 96).

Periodontitis ascertainment

Oral examinations were done every 3-5 years by calibrated dentists. Three different dentists carried out the examinations throughout the period of the DLS (1968-2009) with a study showing that their examinations were reliable and consistent (97). Periodontal measurements including probing pocket depth and gingival recession were first measured in 1981 at 2 interproximal sites per tooth (mesial and distal) and each was scored per tooth the following way: ≤2 millimeters (mm); more than 2 mm but less than or equal to 3 mm; more than 3 mm but less than 5 mm; and ≥5 mm. Clinical attachment loss was calculated ad hoc from measures of pocket depth and gingival recession.

In this study, we applied the 2012 CDC/AAP case definition for periodontitis(67), which categorized the periodontal condition according to the severity and extent of probing depth and clinical attachment loss; severe: more than 2 teeth with >5 mm of CAL and more than one tooth

with >5 mm pocket depth, moderate: more than 2 teeth with > 3 mm of CAL or more than 2 teeth with > 5 mm pocket depth; mild: more than 2 interproximal sites with > 2 mm of CAL and (more than 2 teeth with > 3 mm of pocket depth or one tooth with > 5 mm pocket depth; none: periodontal condition not meeting the previous criteria.

When periodontitis was evaluated as an exposure (i.e., periodontitis-diabetes association), binary variables were constructed with severe or moderate periodontitis collapsed into one category while the mild and none collapsed into another category (severe or moderate vs. mild or none). When periodontitis was evaluated as an outcome, however, (i.e., diabetes-severe periodontitis association), severe periodontitis was estimated in reference to all other forms (severe vs. moderate/mild/none).

Diabetes ascertainment

Diabetes was ascertained in the NAS through self-report during a medical examination and was subsequently coded using ICD-8 codes. As an exposure (i.e., diabetes-severe periodontitis association), diabetes was ascertained by a medical diagnosis of ICD-8 codes based on self-report. However, as an outcome (i.e., periodontitis-diabetes association), diabetes was ascertained by 1) medical diagnosis of ICD-8 codes based on self-report or 2) having diabetes listed as a primary or secondary causes for death.

Cardiovascular disease ascertainment

Cardiovascular disease was ascertained in the NAS through self-report during a medical examination and was subsequently coded using ICD-8 codes. The codes were (410-414) for ischemic heart diseases and codes (432-435) for stroke. In this study, cardiovascular disease was only ascertained as an outcome by 1) medical diagnosis of ICD-8 codes based on self-report of

ischemic heart disease or stroke or 2) having any of these conditions or codes listed as primary or secondary causes for death.

Covariates

Restriction of the DLS participants to white males fully controlled for gender and partially controlled for race/ethnicity. Variables that were controlled for in the analysis phase included age, smoking, and BMI. Age in years was analyzed as a continuous variable.

Data on smoking duration, intensity, and time since quitting smoking were collected and used to calculate the comprehensive smoking index (CSI). The comprehensive smoking index takes into account duration intensity, and time since smoking cessation. It assumes that smoking effects on chronic disease take the form of an exponentially decreasing function (98, 99). Based on the formula here (98, 99), it was calculated as follows: $\sum_{i=1}^{k}\left(1-0.5^{\frac{d_i}{t}}\right)*0.5^{\frac{c_i}{t}}*n_i$ where:

d_i: duration of smoking in i-th period in years.

c_i: time since i-th period in years.

n_i: number of cigarettes per day smoked in i-th period.

t: a half-life parameter in years related to each chronic disease (the exponential decline of smoking effect on the chronic disease). The half-life parameter for chronic periodontitis was estimated to be 1.5 years (98).

The CSI increases with duration and intensity but decreases with the longer cessation of smoking. CSI was analyzed as a continuous variable.

BMI was calculated from weight and height measured using the formula $\text{weight (kg)}/\text{height (m)}^2$ and was analyzed as a continuous variable.

Periodontitis-diabetes association

To study the potential effect of periodontitis on diabetes, our study included DLS participants who were free of diabetes, had at least 2 periodontal examinations between 1981 and 2009, had at least 8 teeth present (excluding third molars) at their baseline periodontal exam, and had data on all covariates at one exam during follow-up. A minimum induction period of 1 year was considered for diabetes therefore, any person who developed diabetes or was censored within their first year of follow-up, was excluded. These exclusions were implemented in order to minimize the potential effects of left truncation, a special form of selection bias, given the chronic nature of both periodontitis and diabetes.

Periodontitis was analyzed as a time-varying exposure. Individuals were followed-up from their first periodontal examination until they developed diabetes, were lost to follow-up, died, developed diabetes within 7 years of their last follow-up, or died within 7-years of their last follow-up. The event of interest was the first diabetic diagnosis. If a person had diabetes listed as a cause of death with no prior diabetic diagnosis, the event was assumed to have occurred at mid-point between the last follow-up and the death date. Censoring was defined as any person who was lost to follow-up, died from reasons other than diabetes, or completed the follow-up period without developing diabetes (administrative censoring). If a person had more than 7 years between any study follow-up cycles then he was artificially censored (N=58). Choosing 7-years as a cut-off point for artificial censoring was based on evaluations of time between movements between categories of the periodontitis exposure i.e. moderate to severe, in the study cohort. Additionally, the choice was not so stringent to the extent that it would make artificial censoring informative (Table 15).

Periodontitis was assumed to change from one category to another at the mid-point of the follow-up interval, while diabetes was assumed to occur at the time of diagnosis or onset, aside from those cases where the cause of death represented the first event.

Periodontitis-CVD association

To study the potential effects of periodontitis on CVD, our study included DLS participants who were free of cardiovascular disease at the study baseline, had at least 2 periodontal examinations between 1981 and 2009, had at least 8 teeth present (excluding third molars) at their baseline periodontal exam, and had data on all covariates at one exam during follow-up. A minimum induction period of 3 years was considered for CVD therefore, any person who developed the outcome, or was censored, within their first 3 years of follow-up, was excluded.

Periodontitis was analyzed as a time-varying exposure. Individuals were followed-up from their first periodontal examination until they developed CVD, were lost to follow-up, died, developed CVD or died within 7 years of their last follow-up. The event was the first CVD diagnosis via self-report or medical diagnosis (ICD-8). If a person had CVD listed as a cause of death then, CVD was assumed to have occurred at the time of death. Censoring was defined as any person who was lost to follow-up, died from reasons other than cardiovascular disease, or completed the follow-up period without developing CVD (administrative censoring). If a person had more than 7 years between any of follow-ups then, he was artificially censored (N=51).

Periodontitis was assumed to change mid-point while diabetes was assumed to occur at the time of diagnosis or onset because the year cardiovascular disease occurred was known, unlike periodontitis.

Diabetes-severe periodontitis association

To study the potential effect of diabetic diagnosis on the risk of severe periodontitis, our study population included DLS participants who were free of severe periodontitis, had at least 2 periodontal examinations between 1981 and 2009, had at least 8 teeth present excluding third molars at their baseline periodontal exam, and had data on all covariates at least in on exam. A minimum induction period of 1 year was implemented for severe periodontitis. Therefore, any person who developed severe periodontitis, or was censored within their first year of follow-up, was excluded.

Diabetes was analyzed as a time-varying exposure. The event was the first severe periodontitis occurrence. Censoring was defined as any person who was lost to follow-up, died, completed the follow-up period without developing severe periodontitis (administrative censoring), or had less than 2 teeth excluding third molars (since the person would no longer be at risk for the definition of severe periodontitis used in this study). Individuals were followed-up from their first periodontal examination until they developed severe periodontitis or were censored.

Statistical analysis

All statistical analysis was carried out using SAS 9.4. Complete case analysis was conducted.

Handling missing data

Periodontitis was assumed to change from one category to another at the mid-point of the follow-up interval. If a person had a missing periodontitis status and came back later into the study within 7 years of their last exam and had the same value of their last recorded periodontitis status then, that value was imputed for the missing exam (no 7-year limit for the diabetes-periodontitis). However, if a person missed an exam and came back within 7 years with a value

for periodontitis that was different than the last recorded value, periodontitis was assumed to change at the mid-point of the follow-up interval and the value was imputed (N=14, 15, and 6) for the periodontitis-diabetes, periodontitis-CVD, and diabetes-periodontitis, respectively.

If a person was missing BMI at any exam, it was imputed by taking the average of the scores immediately preceding and following the missing exam (N=18, 17, and 8) for the periodontitis-diabetes, periodontitis-CVD, and diabetes-periodontitis, respectively. If the last exam was missing, BMI was assumed to have the same value as the last exam (N=7, 7, 2) for the periodontitis-diabetes, periodontitis-CVD, and diabetes-periodontitis, respectively. If a person was missing CSI at the last exam then, it was assumed to have the same value of the last exam (N=2, 3, and 0) for the periodontitis-diabetes, periodontitis-CVD, and diabetes-periodontitis, respectively.

Cox proportional hazards models

Crude and adjusted Cox proportional hazards models were fit with time-varying exposures and with both baseline confounders and time-varying confounders as exposures changed. The choice of the time scale was follow-up time (i.e., time on study). The same models were fit with death from causes other than diabetes and cardiovascular disease as a competing event for the periodontitis-diabetes and periodontitis-CVD associations, respectively. Hazard ratios with their corresponding 95% confidence intervals were reported.

Bias analysis

In order to simulate a confounder into a data set, three parameters were needed: 1) prevalence of the confounder among the exposed, 2) prevalence of the confounder among the non-exposed, and 3) the odds ratio of having the confounder among subjects with the disease compared to those without the disease. Table 16 shows a hypothetical example of a population. Suppose in

a hypothetical population the prevalence of the confounder among the exposed was 0.80 and 0.20 among the non-exposed and that the odds ratio of having the confounder among the diseased compared to the non-diseased was 1.1. Then using the panel A of table 16, the following formula can be used:

$$OR_{confounder-disease} = (A_{c+} * B_{c-}) / (A_{c-} * B_{c+}) = (C_{c+} * D_{c-}) / (C_{c-} * D_{c+}) \quad \text{Formula (1)}$$

Now, the prevalence of the confounder among the exposed was 0.80 and since the non-diseased (200) are as close to the total population as we get, we can know the number of exposed and non-diseased who had the confounder by multiplying 200 by 0.80 to get 160 (panel B of Table 16). Then subtract 160 from 200 to get the number of exposed and non-diseased without the confounder (same can be applied to the non-exposed). By design of formula (1), there is no effect measure modification of the confounder since $(A_{c+} * B_{c-}) / (A_{c-} * B_{c+}) = 1.1$ and $(C_{c+} * D_{c-}) / (C_{c-} * D_{c+}) = 1.1$. Next, we can substitute A_{c+} in equation (1) with $A - A_{c+}$ since $A = A_{c+} + A_{c-}$ and then solve equations 2-4 to get $A_{c-} = 18.52$.

$$1.1 = A_{c+} * 40 / A_{c-} * 160 \quad \text{Equation (1)}$$

$$1.1 = (100 - A_{c-}) * 40 / A_{c-} * 160 \quad \text{Equation (2)}$$

$$176A_{c-} = 4000 - 40A_{c-} \quad \text{Equation (3)}$$

$$216A_{c-} = 4000 \quad \text{Equation (4)}$$

Panels C and D of Table 16 show the rest of the process and the final numbers, from which the prevalence of all four combinations—among the exposed and disease, among the exposed and non-diseased, among the non-exposed diseased, and non-exposed and non-disease—can be calculated. The unmeasured confounder can then be simulated in each

individual through a random binomial function with one trial and a probability of success equal to the prevalence of the confounder and seed of -1 to indicate that it changes every time.

An unmeasured confounder was simulated with different combinations of parameters (confounder-outcome and confounder-exposure associations) at time of failure or censoring (i.e., based on the tabulation of exposure and outcome at the last exam for each person) and then merged with the DLS data to add the confounder to the model and compute the corrected hazard ratios. Each combination was done with 10,000 iterations to account for the random error and the 2.5^{th} and 97.5^{th} percentile interval of corrected hazard ratios for each combination was reported.

Sensitivity analysis

In order to assess the potential impact of varying numbers of teeth across participants, reanalysis with different eligibility criteria of subjects with more than 20 teeth (excluding 3^{rd} molars) present was done to investigate the potential effects of losing teeth on the hazard ratios. This is known as prevalent cohort bias, a special form of left truncation or selection bias, in which subjects who already had periodontitis and lost their teeth due to periodontitis are either not observed or observed as a misclassified non-periodontitis case (85). Therefore, we assumed that restricting based on the number of teeth would minimize any effects of prevalent cohort bias.

Results

Periodontitis-diabetes association

Table 17 shows the baseline distribution of confounders by periodontitis groups. Those participants presenting with severe or moderate periodontitis were older, had fewer teeth, and slightly higher means of the cumulative smoking index and BMI.

The crude incidence rate of diabetes was 1.39 times higher among those with severe or moderate periodontitis compared to those with mild or no periodontitis. Overall, 35% of the

sample died from causes other than diabetes during the follow-up so competing events from death other than diabetes may pose a problem to Cox proportional hazards models below. During follow-up, approximately 16% changed their periodontitis status in the severe moderate periodontitis group while approximately 88% changed their periodontitis status in the other group.

Table 18 shows the results of the crude and adjusted time-dependent Cox proportional hazards models. In the crude model with time-varying periodontitis, those with severe or moderate periodontitis had 1.46 times the hazard (95% CI: 0.79, 2.71) of developing diabetes compared to those without severe or moderate periodontitis. After adjusting for time-dependent confounders (e.g., CSI, BMI), the hazard ratio was 1.32 (95% CI: 0.70, 2.50). After adjusting for baseline confounders (time-fixed), from the hazard ratio was reduced to 1.19 (95% CI: 0.63, 2.26). All three models were fit again but with death from causes other than diabetes as a competing event and the hazard ratios did not change more than 10% compared to the ones without the competing event.

Table 19 shows the corrected hazard ratios of severe or moderate periodontitis on developing diabetes adjusted for baseline confounders after simulating different unmeasured confounders with multidimensional bias analysis. With the presence of a very strong confounder (i.e., OR=2, 1.8, or 0.5 and difference in confounder prevalence between diabetes groups was large), severe or moderate periodontitis may have a protective hazard ratio (<1). In other words, confounding could totally reverse the association between periodontitis and diabetes in the presence of very strong or extreme confounders only.

Presence of a strong confounder (i.e., OR=2, OR=1.8, OR=0.6, and OR=0.5, along with a large or small difference in confounder prevalence between periodontitis groups) can

bring the hazard ratios to almost null effects (< 1.1) in worst case (i.e., the presence of confounding could totally explain the association between periodontitis and diabetes in the presence of a strong confounder). In addition, presence of a moderate confounder (OR=1.1 OR=1.3, OR=1.5, with a large difference in confounder prevalence between periodontitis groups can bring the hazard ratios to almost null effects (< 1.1) in worst case scenarios (i.e., presence of moderate confounder could also partially or totally explain away the association). Other scenarios of unmeasured confounding at least resulted in ≥ 1.1 hazard ratios in their worst-case scenarios (i.e., they can partially explain away the association between periodontitis and diabetes). Severe or moderate periodontitis could have a hazard ratio of developing diabetes as high as 1.8 if those with the confounder had an odds ratio of having diabetes that was 2, 1.8, or 0.5 and the prevalence of the confounder was 90% or 10% among non-severe or moderate periodontitis and 10% or 90% among severe or moderate periodontitis.

Table 20 shows the results of simulating the unmeasured confounders after restriction of the sample to those with > 20 teeth at baseline. In this case, there was a downward shift in all the hazard ratios of all combinations of parameters. Now, the majority of confounders results in potential protective hazard ratios.

Periodontitis-cardiovascular disease association

Table 21 shows the baseline distribution of potential confounders by periodontitis groups. Those with severe or moderate periodontitis were older but had similar BMI and CSI scores, as well as numbers of teeth. Those with severe or moderate periodontitis had 1.15 times the crude incidence rate of developing cardiovascular disease compared to non-severe or moderate periodontitis group. Overall, 23.42% of the sample died from causes other than CVD during the

follow-up so competing events from death other than CVD may pose a problem to Cox proportional hazards models below.

Tables 22 and 23 shows the crude and adjusted time-dependent Cox proportional hazards models of developing cardiovascular disease with and without controlling for diabetes, respectively. The results with and without controlling for diabetes were identical. In the crude model with time-varying periodontitis, those with severe or moderate periodontitis had 1.17 times the hazard of developing cardiovascular disease compared to those without severe or moderate periodontitis(95% CI: 0.78, 1.75). After adjusting time-dependent covariates, the hazard ratio was 1.06 (95% CI: 0.70, 1.60). After adjusting for baseline confounders (time-fixed), the hazard ratio was further reduced (HR=0.96; 95% CI: 0.63, 1.45). All three models were re-fit but with death from causes other than CVD as a competing event and the hazard ratios did not change more than 10% compared to the ones without the competing event.

Tables 24 and 25 show the corrected hazard ratios of severe or moderate periodontitis on developing diabetes adjusted for baseline confounders after simulating different unmeasured confounders with multidimensional bias analysis with and without controlling for diabetes, respectively, which did not impact the results. The majority of confounders simulated could result could either totally explain away the association between periodontitis and CVD or even reverse the association.

Severe or moderate periodontitis could have a hazard ratio of developing diabetes as high as only 1.3 if those with the confounder had an odds ratio of having cardiovascular disease that was 2, 1.8, or 0.5 and the prevalence of the confounder was 90% or 10% among non-severe or moderate periodontitis and 10% or 90% among severe or moderate periodontitis.

Table 26 shows the results of simulating the unmeasured confounders after restriction of the sample to those with > 20 teeth at baseline. In this case, there was a downward shift in all the hazard ratios of all combinations of parameters compared to the original analysis with ≥ 20 teeth. Now, all of the confounders could totally explain away the association and even reverse it.

Diabetes-severe periodontitis association

Table 27 shows the baseline distribution of confounders by diabetes groups. Diabetics had older men, who smoked more, and who had higher BMI. Both groups had the same mean of the number of teeth present at baseline. Diabetics had 1.55 time the crude incidence rate of developing severe periodontitis compared to non-diabetics. Overall, only 1.16% of the sample died during the follow-up so competing events from death would not pose a problem to Cox proportional hazards models below. During follow-up, twenty-seven individuals developed diabetes.

Table 28 shows the crude and adjusted time-dependent Cox proportional hazards models of developing severe periodontitis. In the crude model with time-varying diabetes, diabetics had 1.44 times the hazard of developing severe periodontitis compared to non-diabetics (95% CI: 0.86, 2.44). After adjusting time-dependent covariates, the hazard ratio was 1.34 (95% CI: 0.79, 2.29). After adjusting for baseline confounders (time-fixed), the hazard ratio was further reduced (HR=1.37; 95% CI: 0.80, 2.33).

Table 29 shows the corrected hazard ratios of diabetics on developing severe periodontitis adjusted for baseline confounders after simulating different unmeasured confounders with multidimensional bias analysis. Even with the presence of strong or extreme unmeasured confounders (i.e., OR=2 or 0.5 and difference in confounder prevalence between diabetes groups was large), the confounder could not totally explain away the association

between diabetes and severe periodontitis (i.e., bring the hazard ratio to 1. Furthermore, the confounder could never reverse the association no matter how strong the confounder was..

Presence of extreme confounding can bring the hazard ratios to almost null effects (< 1.1) in worst case scenarios but never to 1. Other scenarios of unmeasured confounding at least resulted in ≥ 1.1 hazard ratios in their worst case scenarios. Diabetes could have a hazard ratio of developing severe periodontitis as high as 4 if those with the confounder had an odds ratio of having severe periodontitis that was 2, 1.8, or 0.5 and the prevalence of the confounder was 90% or 10% among non-diabetics and 10% or 90% among diabetics.

Table 30 shows the results of simulating the unmeasured confounders after restriction of the sample to those with > 20 teeth at baseline. In this case, even in the presence of extreme or very strong unmeasured confounder, the association could not be totally explained away. With restriction to more than 20 teeth, previous combinations of parameters that resulted in a hazard ratio as high as 4, could result in higher hazard ratios.

Discussion

The diabetes-severe periodontitis association was the most robust association between all three and the periodontitis-cardiovascular disease association was the least robust in terms of sensitivity to unmeasured confounders. No matter how strong the confounder was, it could never totally explain away the diabetes-severe periodontitis association. However, confounders could partially, totally explain away, or ever reverse the periodontitis-diabetes and periodontitis-CVD (more so for the periodontitis-CVD). Restriction to individuals with more than 20 teeth, increased the hazard ratios for diabetes-periodontitis but decreased them for periodontitis-diabetes and periodontitis-cardiovascular disease associations.

In the diabetes-periodontitis association, assuming that periodontitis occurred at the mid-point may have introduced misclassified person time but it was most likely non-differential. However, in the absence of accurate ascertainment of periodontitis, it may have been the safest option. Since at least periodontitis (outcome) was assessed objectively, there was unlikely to be a dependent misclassification of diabetes and periodontitis (misclassification of diabetes depends on misclassification of periodontitis or vice-versa). Furthermore, the assumptions for imputing periodontitis status based on the previous and subsequent status may have been violated as a person could still have changed periodontitis at each exam. This may have resulted in non-differential misclassification of person-time that would have biased the estimates towards the null in expectation. However, artificially censoring them instead may have made the censoring more informative and thus could have created more bias than imputing the missing periodontitis values.

In the periodontitis-diabetes association, If a person had diabetes listed as a cause of death, then diabetes was assumed to have occurred at mid-point rather the time of death because it was unlikely that diabetes itself caused immediate death. Choosing 7-years as a cut-off point for artificial censoring was based on the average change of severe and moderate periodontitis in the original cohort dental longitudinal study and also 7 years was not that stringent to the extent it that it would make artificial censoring informative (Table 15). Death from other causes than diabetes was high and could affect the results of Cox model. However, competing events model did not change the results much.

In the periodontitis-CVD association, if a person had CVD listed as a cause of death, then it was assumed to have occurred at the time of death because it was likely that CVD itself caused

immediate death. Death from other causes than CVD was high and could affect the results of Cox model. However, competing events model did not change the results much.

Self-reported diabetes and cardiovascular disease statuses were used rather than objective measures in this study because there was a mismatch between the dates of the Dental Longitudinal Study (periodontitis) cycles and the Normative Aging Study cycles (diabetes and cardiovascular disease) and thus, this would have added another potential non-differential misclassification of exposure that may bias the results towards the null in all associations. However, it may have resulted in less non-differential misclassification bias caused by the self-reported aspect of diabetes and cardiovascular disease and may less likely to result in independent misclassification.

The comprehensive smoking index was used in this study to control for smoking and it offered an advantage over cumulative pack-years. CSI takes intensity, duration, and time since cessation into consideration (unlike cumulative pack-years) (i.e., it treats two people who have the same cumulative pack-years differently according to how long ago they stopped smoking) (98, 99). This may have avoided the residual confounding that could have been introduced by using cumulative pack-years. In addition, body mass index was used and may have resulted in residual confounding. Furthermore, the assumptions for imputing missing values of BMI or CSI may have been violated for the time-varying analysis. This would have resulted in residual confounding in all three associations.

The confounders were simulated at the last exam (i.e., the time of censoring or failure). Thus, they were time-fixed and did not vary in this analysis. This could have resulted in misclassified person-time of the simulated confounder at exams prior to the last one. However, this was a better option than simulating the confounders at baseline (as no person had the

outcome at that time) and also there would be misclassified person-time of the confounders at later exams (i.e., after baseline). Both of these would introduce residual confounding and it may not be known which option had the least residual confounding. Furthermore, in the periodontitis-diabetes and periodontitis-cardiovascular disease associations, deaths as competing events could have modified the simulation of an unmeasured confounder. However, since the hazard ratios from the competing events models did not differ much from the models without the competing events, it was unlikely that death affected the simulations of unmeasured confounders.

This analysis only introduced one unmeasured time-fixed confounder. It is possible that after making it time-varying, the results would change. In addition, there could be several unmeasured confounders and that simply controlling for one would still result in residual confounding in all three associations. In addition, the odds ratios were used instead of risk ratio to determine the relationship between the confounder and the outcome. Odds ratio are known to be a good approximation of the risk ratio in case the risk was low <10% otherwise, the odds ratios tend to overestimate risk ratios (49). So, in this study, the odds ratio may reflect a stronger confounder than if the risk ratio was used.

This analysis intended to simulate the unmeasured confounders among individuals with <20 teeth to investigate the potential of losing teeth on the impact of the unmeasured confounder (i.e., the prevalent cohort bias). The rationale for the stratified analysis was that people who already had periodontitis and lost their teeth may have a different risk of developing periodontitis, diabetes, or cardiovascular disease than those had not already had periodontitis (i.e., prevalent and incident periodontitis, respectively). Unfortunately, this analysis was unable to simulate the unmeasured confounders among participants with less than 20 teeth because small cell counts prohibited simulation of the unmeasured confounder. However, restriction

analysis was still done to see how prevalent cohort bias could impact the effect of the simulated unmeasured confounder. Generally, this resulted in moving the estimates upward for the diabetes-periodontitis association and downward for the periodontitis-diabetes and periodontitis-cardiovascular disease associations.

Confounders that were positively associated with the outcome and negatively associated with the exposure resulted in the same estimates compared to confounders that were negatively associated with the outcome and positively associated with the exposure. This indicated that the needed confounders in this study to provide the full range of confounding should be either positive or negative odds ratios for the association between the confounder and the outcome with all combinations of prevalence (i.e., prevalence higher and lower among exposed). Another option would be to have either higher or lower prevalence among the exposed with all positive and negative odds ratios. This phenomenon resulted because both scenarios introduce the same confounding and thus the same interval of hazard ratios.

Finally, the causal diagrams were based on the literature. There was a chance that the causal diagrams were wrong about the data generating process of the periodontitis-systemic disease associations. Thus, if the causal diagrams were wrong, the results may end up differently.

The limitations of this study include the potential selection bias from differential selection or differential loss to follow-up. Furthermore, the potential violation of the assumptions for handling missing data was a possibility. In addition, the assumption that periodontitis changed at the mid-point of the interval may have been violated. Moreover, self-report diabetes and CVD. Furthermore, the residual confounding from BMI could have been an issue. The potential for time-dependent confounding (treatment-confounder feedback loop) that would bias

the results if the confounder was also on the causal pathway and it was adjusted for. Last, the arbitrarily chosen induction periods could have affected the results.

Conclusion

Presence of one source of unmeasured confounding (one confounder) showed that the diabetes-periodontitis association was robust to it (i.e., no matter how strong the confounder was, it could never totally explain away the association). The periodontitis-diabetes and periodontitis-cardiovascular disease association were less robust to the presence of even weak unmeasured confounding (i.e., weak confounders could totally explain away their associations and strong confounders could even reverse them). There are a lot of factors to consider that may hinder the simulation of unmeasured confounders.

Figures and Tables

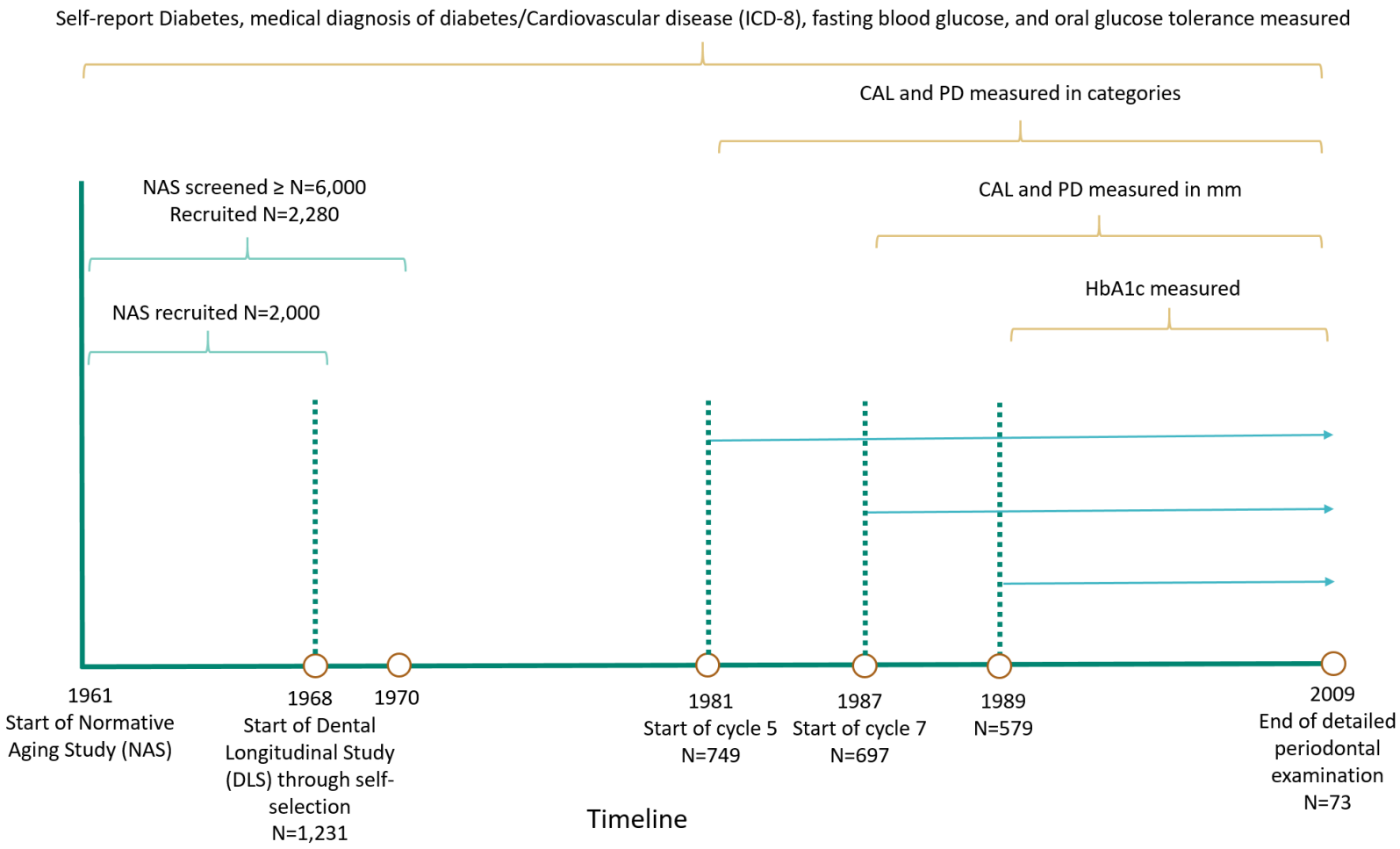

Figure 4. Timeline of the NAS and DLS with periodontitis and diabetes measurements.

Mean change	Follow-up in DLS years rounded to nearest integer									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	N=3	**N=373**	**N=1640**	**N=549**	**N=364**	**N=42**	**N=26**	**N=14**	**N=19**	**N=10**
Any periodontitis (yes/no) (proportion)	0.33	0.19	0.19	0.19	0.16	0.12	0.08	0.08	0.11	0.44
Severe or moderate periodontitis (yes/no) (proportion)	0	0.19	0.20	0.22	0.21	0.15	0.30	0.08	0.11	0.33
Severe periodontitis only (yes/no) (proportion)	0.33	0.20	0.23	0.21	0.30	0.27	0.39	0.33	0.23	0.33

Table 15. Average change of periodontitis between exams by follow-up in years between each exam where N is the number of exams.

A

	Total		With confounder (c+)		Without confounder (c-)	
	Exposed	**Non-exposed**	**Exposed**	**Non-exposed**	**Exposed**	**Non-exposed**
Disease	A	C	A_{c+}	C_{c+}	A_{c-}	C_{c-}
Non-diseased	B	D	B_{c+}	D_{c+}	B_{c-}	D_{c-}

B

	Total		With confounder (c+)		Without confounder (c-)	
	Exposed	**Non-exposed**	**Exposed**	**Non-exposed**	**Exposed**	**Non-exposed**
Disease	100	300	A_{c+}	C_{c+}	A_{c-}	C_{c-}
Non-diseased	200	400	200*0.80=160	400*0.20=80	200-160=40	400-80=320

C

	Total		With confounder (c+)		Without confounder (c-)	
	Exposed	**Non-exposed**	**Exposed**	**Non-exposed**	**Exposed**	**Non-exposed**
Disease	100	300	100-18.52=81.5	C_{c+}	18.5	C_{c-}

Non-diseased	200	400	200*0.80=160	400*0.20=80	200-160=40	400-80=320

D

	Total		**With confounder (c+)**		**Without confounder (c-)**	
	Exposed	**Non-exposed**	**Exposed**	**Non-exposed**	**Exposed**	**Non-exposed**
Disease	100	300	81.5	64.7	18.5	235.3
Non-diseased	200	400	160	80	40	320

Table 16. A hypothetical example of a population in which an unmeasured confounder with the following parameters would be simulated into (prevalence of confounder among exposed=0.80, prevalence of the confounder among the non-exposed=0.20, and the odds ratio of having the confounder among subjects with the disease compared to those without the disease was 1.1).

Variables	Full sample (N=672)	By periodontitis groups: Severe or moderate periodontitis (N=349)	By periodontitis groups: No severe or moderate periodontitis (N=323)
Mean age (SD)	59.60 (8.11)	61.35 (8.35)	57.70 (7.39)
Missing	0	0	0
Mean CSI (SD)	0.55 (1.12)	0.56 (1.14)	0.54 (1.10)
Missing	0	0	0
Mean BMI (SD)	26.44 (3.17)	26.78 (3.27)	26.08 (3.01)
Missing	1	1	0
Mean number of teeth present	22.38 (4.81)	22.05 (4.98)	22.74 (4.59)
Missing	0	0	0
Developed diabetes during follow-up (%)	82 (12.20%)	45 (12.89%)	37 (11.46%)
Person-years of follow-up	9,772.93	4,536.45	5,236.48
Incidence rate of diabetes	0.0084	0.0099	0.0071
Incidence rate ratio		1.39	

Loss to follow-up including deaths below (%)	553		
Death from causes other than diabetes (%)	(82.29%)	293 (83.95%)	260 (80.50%)
	232	123 (35.24%)	109 (33.75%)
	(34.52%)		
Changed periodontitis status at least once over follow-up (%)	339 (50.45%)	56 (16.05%)	283 (87.62%)

SD: standard deviation.

Table 17. Baseline characteristics by periodontitis status of participants who were free of diabetes, had at least 2 periodontal examinations between 1981 and 2009, had at least 8 permanent teeth present during baseline exam, and had data on all covariates at least in on exam (N=672).

Variables	Crude cox model with time-varying periodontitis		Adjusted Cox model with time-varying periodontitis and confounders varying as periodontitis change		Adjusted Cox model with time-varying periodontitis and baseline confounders	
	Hazard ratio	**95% CI**	**Hazard ratio**	**95% CI**	**Hazard ratio**	**95% CI**
Severe or moderate periodontitis						
Yes	1.46	(0.79-2.71)	1.32	(0.70-2.50)	1.19	(0.63-2.26)
No	Ref		Ref		Ref	
Age*			1.00	(0.97-1.03)	1.01	(0.98-1.04)
Cumulative smoking index*			1.16	(0.91-1.49)	1.23	(1.01-1.49)
Body mass index*			1.19	(1.14-1.25)	1.22	(1.16-1.29)

Death from causes other than diabetes as a competing event models	Crude cox model with time-varying periodontitis		Adjusted Cox model with time-varying periodontitis and confounders varying as periodontitis change		Adjusted Cox model with time-varying periodontitis and baseline confounders	
	Hazard ratio	**95% CI**	**Hazard ratio**	**95% CI**	**Hazard ratio**	**95% CI**
Severe or moderate periodontitis						
Yes	1.44	(0.76-2.71)	1.37	(0.70-2.70)	1.26	(0.64-2.48)
No	Ref		Ref		Ref	

CI: Confidence interval.
***** For 1-unit increase.

Table 18. Crude and multivariable time-dependent Cox proportional hazards model of developing diabetes among participants who were free of diabetes, had at least 2 periodontal examinations between 1981 and 2009, had at least 8 permanent teeth present during baseline exam, and had data on all covariates at least in on exam (N=672).

Prevalence of the confounder in periodontitis	Prevalence of the confounder in no periodontitis	$OR_{confounder-diabetes}$									
		2	**1.8**	**1.5**	**1.3**	**1.1**	**0.9**	**0.8**	**0.7**	**0.6**	**0.5**
90%	**10%**	1.01-1.38	1.02-1.41	1.05-1.45	1.07-1.49	1.10-1.55	1.13-1.61	1.15-1.64	1.18-1.70	1.22-1.75	1.27-1.82
80%	**20%**	0.98-1.24	0.99-1.26	1.02-1.31	1.05-1.34	1.08-1.39	1.12-1.45	1.15-1.49	1.17-1.52	1.21-1.57	1.25-1.64
70%	**30%**	1.01-1.19	1.02-1.21	1.05-1.24	1.07-1.27	1.10-1.30	1.14-1.35	1.16-1.37	1.18-1.41	1.21-1.44	1.23-1.49
60%	**40%**	1.06-1.20	1.07-1.20	1.09-1.21	1.11-1.23	1.13-1.25	1.15-1.28	1.17-1.29	1.18-1.31	1.19-1.34	1.19-1.37
40%	**60%**	1.19-1.37	1.18-1.35	1.18-1.32	1.17-1.30	1.16-1.27	1.13-1.25	1.11-1.23	1.10-1.22	1.08-1.21	1.06-1.20
30%	**70%**	1.23-1.48	1.21-1.46	1.19-1.41	1.16-1.38	1.13-1.34	1.10-1.30	1.08-1.28	1.06-1.25	1.03-1.22	1.01-1.20
20%	**80%**	1.25-1.63	1.23-1.60	1.19-1.54	1.16-1.50	1.12-1.44	1.08-1.39	1.06-1.35	1.03-1.32	1.01-1.28	0.98-1.24
10%	**90%**	1.27-1.83	1.24-1.78	1.20-1.72	1.17-1.65	1.13-1.59	1.10-1.54	1.07-1.50	1.06-1.47	1.03-1.42	1.01-1.38

OR: Odds ratio.

Table 19. Parameters for simulating a confounder with different combinations of confounder-diabetes and confounder-periodontitis associations and intervals ranging from (2.5^{th}-97.5^{th}) percentiles of corrected hazard ratios of severe or moderate periodontitis on diabetes when simulating a confounder with 10,000 iterations of each combination (N=672).

Prevalence of the confounder in periodontitis	Prevalence of the confounder in no periodontitis	$OR_{confounder-diabetes}$				
		2	**1.8**	**1.5**	**1.3**	**1.1**
90%	**10%**	0.86-1.26	0.87-1.29	0.90-1.32	0.92-1.35	0.94-1.40
80%	**20%**	0.82-1.10	0.83-1.12	0.86-1.16	0.88-1.19	0.91-1.23
70%	**30%**	0.84-1.03	0.85-1.05	0.88-1.08	0.90-1.10	0.92-1.13
60%	**40%**	0.88-1.03	0.89-1.03	0.91-1.04	0.92-1.05	0.95-1.07
40%	**60%**	1.00-1.19	1.00-1.17	0.99-1.14	0.99-1.12	0.97-1.10
30%	**70%**	1.03-1.30	1.02-1.28	0.99-1.24	0.97-1.21	0.95-1.17
20%	**80%**	1.05-1.45	1.03-1.43	1.00-1.36	0.97-1.32	0.94-1.28
10%	**90%**	1.08-1.65	1.06-1.63	1.02-1.55	0.99-1.50	0.96-1.45

OR: Odds ratio.

Table 20. Parameters for simulating a confounder with different combinations of confounder-diabetes and confounder-periodontitis associations and intervals ranging from (2.5^{th}-97.5^{th}) percentiles of corrected hazard ratios of severe or moderate periodontitis on diabetes when simulating a confounder with 10,000 iterations of each combination among participants who had more than 20 teeth at baseline (N=482).

Variables	Full sample (N=568)	By periodontitis groups	
		Severe or moderate periodontitis (N=280)	No severe or moderate periodontitis (N=288)
Mean age (SD)	59.25 (8.08)	61.17 (8.44)	57.38 (7.25)
Missing	0	0	0
Mean CSI (SD)	0.53 (1.11)	0.53 (1.13)	0.53 (1.09)
Missing	0	0	0
Mean BMI (SD)	26.37 (3.16)	26.61 (3.15)	26.15 (3.16)
Missing	1	1	0
Mean number of teeth present	22.49 (4.72)	22.06 (5.05)	22.91 (4.34)
Missing	0	0	0
Developed cardiovascular disease during follow-up (%)	175 (30.81%)	86 (30.71%)	89 (30.90%)
Person-years of follow-up	8,191.24	3,744.58	4,446.66
Incidence rate of cardiovascular disease	0.0214	0.0230	0.0200

Incidence rate ratio		1.15	
Loss to follow-up including death (%)	369 (64.96%)	186 (66.43%)	183 (63.54%)
Death (%)	133 (23.42%)	70 (25.00%)	63 (21.88%)
Changed periodontitis status at least once over follow-up (%)	303 (53.35%)	47 (16.79%)	256 (88.89%)

SD: standard deviation.

Table 21. Baseline characteristics by periodontitis status of participants who were free of cardiovascular disease, had at least 2 periodontal examinations between 1981 and 2009, had at least 8 permanent teeth present during baseline exam, and had data on all covariates at least in on exam (N=568).

Variables	Crude cox model with time-varying periodontitis		Adjusted Cox model with time-varying periodontitis and confounders varying as periodontitis change		Adjusted Cox model with time-varying periodontitis and baseline confounders	
	Hazard ratio	**95% CI**	**Hazard ratio**	**95% CI**	**Hazard ratio**	**95% CI**
Severe or moderate periodontitis						
Yes	1.17	(0.78-1.75)	1.06	(0.70-1.60)	0.96	(0.63-1.45)
No	Ref		Ref		Ref	
Age*			1.02	(1.00-1.04)	1.04	(1.02-1.06)
Comprehensive smoking index*			0.88	(0.69-1.11)	0.97	(0.83-1.13)
Body mass index*			1.04	(1.00-1.08)	1.07	(1.02-1.12)

Death from causes other than diabetes as a competing event models	Crude cox model with time-varying periodontitis		Adjusted Cox model with time-varying periodontitis and confounders varying as periodontitis change		Adjusted Cox model with time-varying periodontitis and baseline confounders	
	Hazard ratio	**95% CI**	**Hazard ratio**	**95% CI**	**Hazard ratio**	**95% CI**
Severe or moderate periodontitis						
Yes	1.16	(0.77-1.73)	1.08	(0.72-1.61)	1.02	(0.68-1.54)
No	Ref		Ref		Ref	

CI: Confidence interval.
***** For 1-unit increase.

Table 22. Crude and multivariable time-dependent Cox proportional hazards model of developing cardiovascular disease among participants who were free of cardiovascular disease, had at least 2 periodontal examinations between 1981 and 2009, had at least 8 permanent teeth present during baseline exam, and had data on all covariates at least in on exam without controlling for diabetes (N=568).

Variables	Crude cox model with time-varying periodontitis		Adjusted Cox model with time-varying periodontitis and confounders varying as periodontitis change		Adjusted Cox model with time-varying periodontitis and baseline confounders	
	Hazard ratio	**95% CI**	**Hazard ratio**	**95% CI**	**Hazard ratio**	**95% CI**
Severe or moderate periodontitis						
Yes	1.17	(0.78-1.75)	1.06	(0.70-1.59)	0.96	(0.63-1.45)
No	Ref		Ref		Ref	
Age*			1.02	(1.00-1.04)	1.04	(1.02-1.06)
Comprehensive smoking index*			0.88	(0.69-1.11)	0.97	(0.83-1.14)
Body mass index*			1.04	(0.99-1.08)	1.06	(1.01-1.12)
Diabetes						
Yes			1.38	(0.79-2.40)	1.69	(0.69-4.15)
No			Ref		Ref	

Death from causes other than diabetes as a competing event models	Crude cox model with time-varying periodontitis		Adjusted Cox model with time-varying periodontitis and confounders varying as periodontitis change		Adjusted Cox model with time-varying periodontitis and baseline confounders	
	Hazard ratio	**95% CI**	**Hazard ratio**	**95% CI**	**Hazard ratio**	**95% CI**
Severe or moderate periodontitis						
Yes	1.16	(0.77-1.73)	1.08	(0.72-1.61)	1.02	(0.68-1.54)
No	Ref		Ref		Ref	

CI: Confidence interval.
***** For 1-unit increase.

Table 23. Crude and multivariable time-dependent Cox proportional hazards model of developing diabetes among participants who were free of diabetes, had at least 2 periodontal examinations between 1981 and 2009, had at least 8 permanent teeth present during baseline exam, and had data on all covariates at least in on exam with controlling for diabetes (N=568).

Prevalence of the confounder in periodontitis	Prevalence of the confounder in no periodontitis	$OR_{confounder-cardiovascular\ disease}$									
		2	**1.8**	**1.5**	**1.3**	**1.1**	**0.9**	**0.8**	**0.7**	**0.6**	**0.5**
90%	**10%**	0.82-1.05	0.83-1.07	0.85-1.11	0.87-1.13	0.89-1.17	0.92-1.21	0.94-1.24	0.97-1.27	0.99-1.31	1.03-1.35
80%	**20%**	0.79-0.97	0.81-0.99	0.83-1.02	0.85-1.05	0.88-1.08	0.91-1.12	0.93-1.15	0.96-1.18	0.99-1.21	1.02-1.25
70%	**30%**	0.82-0.95	0.83-0.96	0.85-0.98	0.87-1.00	0.89-1.03	0.92-1.06	0.94-1.08	0.95-1.10	0.97-1.13	0.99-1.16
60%	**40%**	0.85-0.96	0.87-0.96	0.88-0.96	0.90-0.97	0.92-0.99	0.94-1.01	0.95-1.02	0.95-1.04	0.96-1.06	0.96-1.08
40%	**60%**	0.95-1.08	0.96-1.07	0.95-1.05	0.95-1.03	0.93-1.01	0.91-0.99	0.90-0.98	0.89-0.97	0.87-0.96	0.86-0.96
30%	**70%**	0.99-1.16	0.98-1.14	0.96-1.11	0.94-1.08	0.92-1.06	0.89-1.02	0.88-1.01	0.86-0.99	0.84-0.96	0.82-0.94
20%	**80%**	1.02-1.25	1.00-1.23	0.97-1.19	0.94-1.15	0.91-1.12	0.88-1.08	0.86-1.05	0.84-1.03	0.82-1.00	0.79-0.97
10%	**90%**	1.03-1.36	1.01-1.33	0.97-1.28	0.95-1.25	0.92-1.21	0.89-1.17	0.87-1.14	0.86-1.11	0.84-1.09	0.81-1.05

OR: Odds ratio.

Table 24. Parameters for simulating a confounder with different combinations of confounder-cardiovascular disease and confounder-periodontitis associations and intervals ranging from (2.5^{th}-97.5^{th}) percentiles of corrected hazard ratios of severe or moderate periodontitis on cardiovascular disease when simulating a confounder with 10,000 iterations of each combination without controlling for diabetes (N=568).

Prevalence of the confounder in periodontitis	Prevalence of the confounder in no periodontitis	$OR_{confounder-cardiovascular\ disease}$									
		2	**1.8**	**1.5**	**1.3**	**1.1**	**0.9**	**0.8**	**0.7**	**0.6**	**0.5**
90%	**10%**	0.82-1.06	0.83-1.07	0.85-1.11	0.87-1.13	0.89-1.17	0.93-1.22	0.95-1.24	0.97-1.28	0.99-1.31	1.03-1.35
80%	**20%**	0.79-0.97	0.81-0.99	0.83-1.02	0.85-1.05	0.88-1.08	0.91-1.12	0.93-1.15	0.96-1.18	0.99-1.21	1.02-1.25
70%	**30%**	0.82-0.95	0.83-0.96	0.85-0.98	0.87-1.00	0.89-1.03	0.92-1.06	0.94-1.08	0.96-1.11	0.97-1.13	0.99-1.16
60%	**40%**	0.85-0.96	0.87-0.96	0.88-0.97	0.90-0.97	0.92-0.99	0.94-1.01	0.95-1.02	0.95-1.04	0.96-1.06	0.96-1.08
40%	**60%**	0.96-1.08	0.96-1.07	0.95-1.05	0.95-1.03	0.93-1.01	0.91-0.99	0.90-0.98	0.89-0.97	0.87-0.96	0.86-0.96
30%	**70%**	0.99-1.16	0.98-1.14	0.96-1.11	0.94-1.09	0.92-1.06	0.89-1.03	0.88-1.01	0.86-0.99	0.84-0.96	0.82-0.95
20%	**80%**	1.02-1.25	1.00-1.23	0.97-1.19	0.94-1.16	0.91-1.12	0.88-1.08	0.86-1.05	0.84-1.03	0.82-1.00	0.79-0.97
10%	**90%**	1.03-1.36	1.01-1.33	0.98-1.28	0.95-1.25	0.92-1.21	0.89-1.17	0.88-1.15	0.86-1.11	0.84-1.09	0.82-1.06

OR: Odds ratio.

Table 25. Parameters for simulating a confounder with different combinations of confounder-cardiovascular disease and confounder-periodontitis associations and intervals ranging from (2.5^{th}-97.5^{th}) percentiles of corrected hazard ratios of severe or moderate periodontitis on cardiovascular disease when simulating a confounder with 10,000 iterations of each combination with controlling for diabetes (N=568).

Prevalence of the confounder in periodontitis	Prevalence of the confounder in no periodontitis	$OR_{confounder-cardiovascular\ disease}$									
		With controlling for diabetes					Without controlling for diabetes				
		2	1.8	1.5	1.3	1.1	2	1.8	1.5	1.3	1.1
90%	**10%**	0.73-0.97	0.74-0.99	0.76-1.02	0.78-1.04	0.80-1.07	0.73-0.96	0.74-0.98	0.76-1.01	0.77-1.04	0.79-1.07
80%	**20%**	0.70-0.87	0.71-0.89	0.73-0.92	0.75-0.94	0.77-0.97	0.70-0.87	0.71-0.89	0.73-0.92	0.75-0.94	0.77-0.97
70%	**30%**	0.71-0.84	0.72-0.85	0.74-0.87	0.76-0.89	0.78-0.91	0.71-0.84	0.72-0.85	0.74-0.87	0.76-0.89	0.78-0.91
60%	**40%**	0.74-0.84	0.75-0.85	0.77-0.85	0.78-0.86	0.80-0.87	0.74-0.84	0.75-0.84	0.77-0.85	0.78-0.86	0.79-0.87
40%	**60%**	0.83-0.96	0.83-0.94	0.83-0.93	0.82-0.91	0.81-0.89	0.83-0.95	0.83-0.94	0.83-0.92	0.82-0.91	0.81-0.89
30%	**70%**	0.86-1.03	0.85-1.02	0.84-0.99	0.82-0.97	0.80-0.94	0.86-1.03	0.85-1.01	0.84-0.99	0.82-0.96	0.80-0.94
20%	**80%**	0.88-1.13	0.87-1.11	0.84-1.07	0.82-1.04	0.80-1.01	0.88-1.12	0.87-1.10	0.84-1.06	0.82-1.03	0.80-1.01
10%	**90%**	0.90-1.23	0.89-1.21	0.86-1.17	0.84-1.15	0.82-1.11	0.90-1.22	0.88-1.20	0.86-1.16	0.84-1.14	0.82-1.10

OR: Odds ratio.

Table 26. Parameters for simulating a confounder with different combinations of confounder-cardiovascular disease and confounder-periodontitis associations and intervals ranging from (2.5^{th}-97.5^{th}) percentiles of corrected hazard ratios of severe or moderate periodontitis on cardiovascular disease when simulating a confounder with 10,000 iterations of each combination among participants with more than 20 teeth at baseline (N=415).

Variables		By diabetes groups	
	Full sample (N=521)	**Diabetic (N=14)**	**Non-diabetic (N=507)**
Mean age (SD)	58.91 (7.71)	62.29 (5.01)	58.82 (7.76)
Missing	0	0	0
Mean CSI (SD)	0.49 (1.06)	0.39 (0.97)	0.49 (1.06)
Missing	0	0	0
Mean BMI (SD)	26.43 (3.19)	29.03 (4.42)	26.36 (3.12)
Missing	1	1	0
Mean number of teeth present	22.58 (4.68)	22.71 (3.24)	22.57 (4.72)
Missing	0	0	0
Developed severe periodontitis during follow-up (%)	264 (50.67%)	7 (50.00%)	257 (50.69%)
Person-years of follow-up	5,321.22	92.68	5,228.54
Incidence rate of severe periodontitis	0.0496	0.0755	0.0492
Incidence rate ratio		1.53	
Loss to follow-up including deaths (%)	233 (44.72%)	7 (50.00%)	226 (44.58%)

Death (%)	6 (1.15%)	0	6 (1.18%)
Developed diabetes over follow-up (%)	27 (5.18%)		27 (5.33%)

SD: standard deviation.

Table 27. Baseline characteristics by diabetes status of participants who were free of severe periodontitis, had at least 2 periodontal examinations between 1981 and 2009, had at least 8 permanent teeth present during baseline exam, and had data on all covariates at least in on exam (N=521).

Variables	Crude cox model with time-varying diabetes		Adjusted Cox model with time-varying diabetes and confounders varying as diabetes change		Adjusted Cox model with time-varying diabetes and baseline confounders	
	Hazard ratio	**95% CI**	**Hazard ratio**	**95% CI**	**Hazard ratio**	**95% CI**
Diabetes						
Yes	1.45	(0.86-2.45)	1.31	(0.76-2.24)	1.34	(0.78-2.30)
No	Ref		Ref		Ref	
Age*			1.04	(1.02-1.06)	1.04	(1.02-1.06)
Cumulative smoking index*			1.30	(1.16-1.47)	1.23	(1.11-1.37)
Body mass index*			1.02	(0.99-1.06)	1.02	(0.98-1.06)

CI: Confidence interval.
***** For 1-unit increase.

Table 28. Crude and multivariable time-dependent Cox proportional hazards model of developing severe periodontitis among participants who were free of severe periodontitis, had at least 2 periodontal examinations between 1981 and 2009, had at least 8 permanent teeth present during baseline exam, and had data on all covariates at least in on exam (N=521).

Prevalence of the confounder among diabetics	Prevalence of the confounder among non-diabetics	$OR_{confounder-severe\ periodontitis}$									
		2	**1.8**	**1.5**	**1.3**	**1.1**	**0.9**	**0.8**	**0.7**	**0.6**	**0.5**
90%	**10%**	1.05-1.60	1.10-1.71	1.17-1.91	1.24-2.08	1.32-2.39	1.44-2.73	1.50-3.01	1.57-3.40	1.67-3.78	1.76-4.34
80%	**20%**	1.03-1.35	1.06-1.40	1.12-1.50	1.17-1.59	1.22-1.72	1.31-1.90	1.36-2.02	1.41-2.16	1.46-2.36	1.54-2.59
70%	**30%**	1.07-1.31	1.10-1.33	1.14-1.39	1.19-1.44	1.23-1.52	1.29-1.63	1.33-1.69	1.36-1.78	1.39-1.89	1.41-2.01
60%	**40%**	1.14-1.37	1.16-1.37	1.20-1.36	1.23-1.38	1.26-1.42	1.30-1.48	1.32-1.52	1.32-1.58	1.32-1.63	1.31-1.71
40%	**60%**	1.31-1.71	1.32-1.66	1.32-1.59	1.32-1.53	1.30-1.48	1.26-1.42	1.24-1.39	1.21-1.37	1.17-1.36	1.14-1.37
30%	**70%**	1.41-2.02	1.40-1.94	1.37-1.81	1.34-1.73	1.29-1.62	1.23-1.51	1.20-1.46	1.16-1.40	1.11-1.35	1.07-1.31
20%	**80%**	1.53-2.59	1.50-2.44	1.43-2.22	1.37-2.08	1.31-1.90	1.23-1.72	1.19-1.63	1.13-1.52	1.08-1.44	1.03-1.35
10%	**90%**	1.77-4.37	1.72-4.01	1.61-3.48	1.52-3.11	1.43-2.73	1.33-2.34	1.26-2.16	1.20-1.98	1.13-1.78	1.05-1.60

OR: Odds ratio.

Table 29. Parameters for simulating a confounder with different combinations of confounder-severe periodontitis and confounder-diabetes associations and intervals ranging from (2.5^{th}-97.5^{th}) percentiles of corrected hazard ratios of diabetes on severe periodontitis when simulating a confounder with 10,000 iterations of each combination (N=521).

Prevalence of the confounder among diabetics	Prevalence of the confounder among non-diabetics	$OR_{confounder-severe\ periodontitis}$				
		2	**1.8**	**1.5**	**1.3**	**1.1**
90%	**10%**	1.15-1.86	1.19-1.99	1.26-2.24	1.33-2.51	1.42-2.88
80%	**20%**	1.12-1.53	1.15-1.60	1.21-1.72	1.27-1.83	1.33-2.00
70%	**30%**	1.18-1.48	1.20-1.50	1.25-1.56	1.29-1.63	1.35-1.74
60%	**40%**	1.25-1.57	1.27-1.56	1.31-1.54	1.34-1.56	1.39-1.60
40%	**60%**	1.45-2.02	1.45-1.95	1.46-1.85	1.45-1.76	1.43-1.68
30%	**70%**	1.54-2.42	1.53-2.31	1.50-2.13	1.47-1.99	1.42-1.87
20%	**80%**	1.66-3.22	1.62-2.98	1.55-2.69	1.50-2.46	1.42-2.22
10%	**90%**	1.89-5.94	1.83-5.34	1.72-4.52	1.64-3.97	1.53-3.35

OR: Odds ratio.

Table 30. Parameters for simulating a confounder with different combinations of confounder-severe periodontitis and confounder-diabetes associations and intervals ranging from (2.5^{th}-97.5^{th}) percentiles of corrected hazard ratios of diabetes on severe periodontitis when simulating a confounder with 10,000 iterations of each combination among participants with more than 20 teeth at baseline (N=380).

Chapter 5: Conclusion

This dissertation aimed to investigate some potential sources of systematic error in the estimation of periodontitis associations with diabetes and cardiovascular disease using diabetes and cardiovascular disease as vessels. The potential errors investigated were misclassification of periodontitis due to case definitions or partial-mouth measurements. Another source of error investigated was confounding (common cause) of both periodontitis and diabetes and cardiovascular disease that may explain their associations.

Chapter 2/Study 1 looked into the impact of using different cut-off points for the required number of teeth with a certain level of CAL or PD, as well as the cut-off points for clinical severity on periodontitis associations with diabetes and cardiovascular disease. Specifically, investigate the presence of risk thresholds of diabetes and cardiovascular disease for periodontitis via different classifications of periodontitis as well as how these classifications affect diabetes association with periodontitis.

It concluded that the effects of clinical severity on the odds ratios were association dependent. Clinical measures and extent did not depend on the association. The direction of the odds ratios among 1-15 depended on the association. Interpretation of these results was limited by the lack of a gold standard (with respect to the type of bias).

Chapter 3/Study 2 looked into the impact of periodontitis misclassification resulting from use of several partial mouth protocols on its association as an exposure with diabetes and cardiovascular disease and as an outcome with diabetes in relation to full-mouth measurements.

It concluded that the amount of bias due to PMPs may be dependent on the size of the underlying association. The choice of protocol did not seem to play a major role in the amount of bias (number of teeth vs. type of tooth included). Percent relative bias was generally less than

10% for the severe categories while it tended to exceed 10% for moderate categories. Bias was increased among people with fewer teeth and the direction depended on the outcome. Mean clinical attachment loss resulted in minimal bias. Overall, bias due to PMP would not have changed the main conclusions when severe periodontitis was under study.

Chapter 4/Study 3 looked into the effects of unmeasured confounders on the associations between periodontitis and diabetes and cardiovascular disease. Presence of one source of unmeasured confounding (one confounder) showed that the diabetes-periodontitis association was robust to it. Unmeasured confounders could not totally explain away the diabetes-severe periodontitis association. However, they could totally explain the periodontitis-diabetes or periodontitis-CVD associations or even reverse them. There are a lot of factors to consider that may hinder the simulation of an unmeasured confounder.

These sources played a role in the periodontitis-diabetes, periodontitis-CVD, and diabetes-periodontitis associations. The associations were affected differently by each source. Some of these sources of systematic errors may change the conclusions of the associations.

CHAPTER 3 APPENDIX

List of Appendix Tables

Chapter 3: Appendix

Methods

Fixed selection

In addition to the Community Periodontal Index of Treatment Needs (CPITN) and the Ramfjörd protocols, The following six protocols were considered: (i) all maxillary teeth (Mx), (ii) all mandibular teeth (Mn), (iii) all teeth on the right side (Rt), (iv) all teeth on the left side (Lt), (v) Half-mouth with maxillary right and mandibular left contralateral sides (Mx Rt & Mn Lt), (vi) Half-mouth with maxillary left and mandibular right contralateral sides (Mx Lt & Mn Rt).

Random selection

In addition to the random half-mouth protocol, the following two protocols were considered: the random maxillary/mandibular teeth (Mx / Mn) in which the maxillary teeth were selected if the binomial value was 1, and the mandibular teeth if the value was 0 and the random right/left side (Rt / Lt) in which the right side was selected if the value was 1, and the left side if the value was 0.

Results

The sample eligible for this analysis was 10,138. The full eligible sample had a mean age of 49.70 (with a standard error of 0.22), was 49.20% males, and the majority had non-Hispanic white (67.62%). Diabetes was in 9.35% and cardiovascular disease was in 5.54% of the sample. The prevalence of severe, moderate, mild, and no periodontitis were 7.80%, 29.00%, 4.42%, and 58.77%, respectively. The sample had missing diabetes status, cardiovascular disease status,

pack-years, and waist to height ratio that were 0.03%, and 0.18%, 2.23%, 2.65%, respectively. Tables 9 shows the distribution of the variables by periodontitis and by diabetes groups.

Full-mouth adjusted analysis are shown in Table 10. When periodontitis was an exposure and CDC/AAP definition was used, those with severe periodontitis had 1.09 (95% CI: 0.77, 1.54) times the odds of having diabetes compared to those with mild or no periodontitis. Moderate periodontitis had 1.30 (95% CI: 1.07, 1.57) times the odds of having diabetes compared to those with mild or no periodontitis. For mean CAL, each 1 mm increase in clinical attachment loss was associated with a 6% increase in the odds (OR=1.06) of having diabetes (95% CI: 0.98, 1.14). Furthermore, each 1 mm increase in clinical attachment loss was associated with a 14% increase in the odds of having cardiovascular disease (OR 95% CI: 1.06, 1.22).

In periodontitis as CDC/AAP outcome, diabetics had 1.01 times the odds of having severe periodontitis (as opposed to mild or no periodontitis) compared to non-diabetics with a compatibility interval ranging from 0.72-1.43. Diabetics had 1.25 times the odds of having moderate periodontitis (as opposed to mild or no periodontitis) compared to non-diabetics with a compatibility interval ranging from 1.02-1.52. For mean CAL, diabetics had 0.05 higher mean of clinical attachment loss compared to non-diabetics with a compatibility interval ranging from -0.04 to 0.14.

Exposure misclassification

Categorical Periodontitis

Percent relative bias was less than 10% for the majority of PMPs when estimating the effects of severe periodontitis on diabetes and CVD (Table 11). Additionally, bias was mixed in

direction (i.e., exposure misclassification resulted in underestimation or overestimation in some cases). The size of the effect of severe periodontitis was larger when CVD was the outcome compared to diabetes (Table 12). In addition, the estimates were mixed in direction for diabetes but were positive for CVD.

Generally, there was no trend in the percent relative bias of the effect of severe periodontitis across all PMPs (Table 11). In addition, there was no trend when comparing the average percent relative bias of fixed 14 teeth (Mx, Mn, Rt, Lt, Mx Rt & Mn Lt, or Mx Lt, & Mn Rt) to 10 teeth (CPITN) and 6 teeth (Ramfjörd) protocols. However, average percent relative bias of random 14 teeth protocols was lower than average of fixed 14 teeth protocols.

When subjects were stratified according to the number of teeth at the time of exam, findings were similar for those with greater than 15 teeth whereas for those with 2- 15 teeth, bias was greater (Table 11). The direction of bias depended on the outcome in both strata. Furthermore, the impact of the protocol chosen on percent relative bias did not differ across strata of teeth.

More PMPs exceeded 10% relative bias when estimating the effects of moderate periodontitis on diabetes and CVD compared to severe periodontitis yet generally, majority of PMPs still had <10% relative bias (Table 11). The direction of bias was generally to the null (underestimation). Furthermore, the effects of moderate periodontitis on diabetes generally had more bias compared to the effects of severe periodontitis but this did not apply to CVD. Estimates of moderate periodontitis were larger when compared to severe periodontitis for diabetes but were the same for CVD and the direction of estimates were positive for both outcomes (Table 12).

The Ramfjörd protocol (6 teeth) had the largest bias among all PMPs (Table 11). However, there was no trend with respect to number of teeth (6, 10, and 14). Unlike severe periodontitis, the averages of random and fixed 14 teeth protocols were the same.

Stratified analysis by number of teeth of the effects moderate periodontitis showed the same findings as the effects of severe periodontitis in terms of extent and dependency of direction on the outcome (Table 11). However, the impact of the protocol chosen on percent relative differed across strata of teeth.

Continuous Periodontitis (mean CAL)

Percent relative bias was less than 1% and 5% when estimating the effects of a 1 mm increase in CAL on diabetes and CVD, respectively (Table 11). Moreover, the direction was generally to the null. The effect sizes of mean CAL was larger on CVD compared to diabetes but the direction was positive in both outcomes (Table 12). There were no trends when comparing different partial-mouth protocols.

Stratified analysis by number of teeth the effects of mean CAL resulted in the same findings of categorical periodontitis in terms of having more bias among those with fewer teeth and dependency of direction on the outcome (Table 11). However, mean CAL was affected less by number of teeth compared to categorical periodontitis.

When all previous analysis was restricted to ≥ 8, ≥ 20, or all 28 teeth. The percent relative bias was mixed in direction and extent (i.e., no trend with more restriction) (Table 11). However, restriction to 28 teeth generally resulted in the highest bias. Moreover, severe periodontitis had more bias than moderate periodontitis when restricting to all teeth. In addition, the direction of bias depended on the outcome. Furthermore, the Ramfjörd protocol had the highest bias. Last,

bias of mean CAL was affected to a lesser degree by restriction compared to categorical periodontitis.

Outcome misclassification

Categorical Periodontitis

Percent relative bias was less than 10% when estimating the effects of diabetes on severe periodontitis and the direction was generally mixed (Table 13). The size of the effect of diabetes on severe periodontitis was mixed in direction across all PMPs (Table 14).

Generally, there was no trend in the percent relative bias of the effect of severe periodontitis across all PMPs (Table 13). In addition, there was no trend when comparing the average percent relative bias of fixed 14 teeth (Mx, Mn, Rt, Lt, Mx Rt & Mn Lt, or Mx Lt, & Mn Rt) to 10 teeth (CPITN) and 6 teeth (Ramfjörd) protocols. However, average percent relative bias of random 14 teeth protocols was lower than average of fixed 14 teeth protocols.

Stratified analysis of the effect of diabetes on severe periodontitis according to the number of teeth had the same findings for those with greater than 15 teeth whereas for those with 2- 15 teeth, bias was greater and the direction was generally to the null (Table 13). In addition, the Ramfjörd protocol had the highest bias in both strata. Furthermore, the impact of the protocol chosen on percent relative bias did not differ across strata of teeth.

Effects of diabetes on moderate periodontitis, unlike its effects on severe periodontitis, generally had more than 10% relative bias and the direction was to the null (Table 13). In addition, effects of diabetes on moderate periodontitis generally had more bias compared to its effects on severe periodontitis. Estimates of diabetes on moderate periodontitis were positive for all PMPs (Table 14).

In terms of trends among PMPs, effects of diabetes on moderate periodontitis had the same findings as effects on severe periodontitis except that bias was not different between averages of fixed and random 14 teeth protocols.

Stratified analysis by number of teeth of the effects of diabetes on moderate periodontitis had the same findings as its effects on severe periodontitis in terms of extent but the direction was to the null in both strata of teeth (Table 13). However, the impact of the protocol chosen on percent relative differed across strata of teeth.

Continuous Periodontitis (mean CAL)

Percent relative bias was more than 10% when estimating the effects of diabetes on mean CAL and the direction was to the null (Table 13). Mean CAL had more bias than categorical periodontitis. The effect size of diabetes on mean CAL was positive (Table 14). Trends comparing different PMPs had the same finding as the effects of diabetes on severe periodontitis. Stratified analysis by number of teeth of the effects of diabetes on mean CAL showed that bias was higher for those with 2-15 teeth and the direction was away from the null (Table 13).

The percent relative bias was mixed in direction and extent (i.e., no trend with more restriction) (Table 13). However, restriction to 28 teeth generally resulted in the highest bias. Moreover, effects of diabetes on severe periodontitis had more bias than its effects on moderate periodontitis when restricting to all teeth. Furthermore, the Ramfjörd protocol had the highest bias. Last, bias of mean CAL was affected to about the same degree by restriction compared to categorical periodontitis.

Tables

Partial-mouth protocol (number of teeth)	Exposure misclassification					
	Periodontitis - Diabetes			Periodontitis – Cardiovascular disease		
	Severe[ϖ]	Moderate[§]	Mean CAL	Severe[ϖ]	Moderate[§]	Mean CAL
Mx (14)						
≥ 2 teeth	3.67%	-11.54%	0.00%	1.47%	-3.65%	-4.39%
> 15 / ≤ 15 teeth	6.60% / -15.79%	-5.04% / -38.28%	0.95% / 0.96%	0.00% / 89.23%	0.00% / 42.19%	0.84% / 9.78%
≥ 8 teeth	0.92%	-14.50%	-2.78%	2.22%	1.49%	-2.59%
≥ 20 teeth	6.42%	-6.56%	0.94%	3.75%	1.42%	0.80%
28 teeth	81.48%	16.42%	12.50%	-39.22%	24.86%	-11.48%
Mn (14)						
≥ 2 teeth	-2.75%	-4.62%	-0.94%	-1.47%	0.73%	-0.88%
> 15 / ≤ 15 teeth	5.66% / -34.59%	-5.04% / -15.79%	-0.95% / -0.96%	1.43% / -7.69%	-3.43% / 18.75%	-0.84% / 2.17%
≥ 8 teeth	-0.92%	-4.58%	2.22%	2.22%	-0.75%	-1.72%
≥ 20 teeth	0.92%	-6.56%	-1.89%	7.50%	-7.09%	-3.20%

28 teeth	42.59%	-20.90%	-6.25%	-20.78%	-3.78%	5.74%
Rt (14)						
≥ 2 teeth	-3.67%	-2.31%	-0.94%	-10.29%	-2.19%	0.88%
> 15 / ≤ 15 teeth	-2.83% / -20.30%	2.52% / -33.01%	0.00% / -1.92%	-12.14% /	0.00% / 1.56%	0.00% / 5.43%
≥ 8 teeth	-2.75%	-3.05%	-0.93%	13.85%	-2.24%	0.86%
≥ 20 teeth	-2.75%	0.00%	-0.94%	-8.89%	-4.26%	0.00%
28 teeth	42.59%	-16.42%	-2.50%	-8.13%	-7.03%	-0.82%
				-64.31%		
Lt (14)						
≥ 2 teeth	-4.59%	-10.00%	0.00%	5.88%	-2.19%	-1.75%
> 15 / ≤ 15	-0.94% / -31.58%	-5.88% / -35.89%	0.00% / 0.96%	5.71% / 21.54%	4.79% / -10.94%	-0.84% / 0.00%
teeth	-6.42%	-11.45%	0.00%	7.41%	3.73%	-0.86%
≥ 8 teeth	-2.75%	-8.20%	-0.94%	7.50%	9.93%	-0.80%
≥ 20 teeth	44.44%	17.91%	2.50%	30.59%	-30.27%	-0.82%
28 teeth						

Mx Rt & Mn Lt						
(14)	4.59%	-10.77%	0.00%	1.47%	-4.38%	-0.88%
≥ 2 teeth	3.77% / -9.02%	-5.04% / -44.98%	0.95% / -0.96%	2.14% / 21.54%	0.68% / -4.69%	1.68% / -1.09%
> 15 / ≤ 15 teeth	2.75%	-12.21%	0.93%	2.96%	-0.75%	0.86%
≥ 8 teeth	0.92%	-4.10%	0.00%	7.50%	0.00%	0.80%
≥ 20 teeth	29.63%	2.99%	3.75%	26.67%	-47.03%	-2.46%
28 teeth						
Mx Lt & Mn Rt						
(14)	-15.60%	-6.92%	-0.94%	-1.47%	0.00%	0.00%
≥ 2 teeth	-12.26% / -	0.00% / -45.45%	-0.95% / 0.00%	-5.00% / 23.08%	1.37% / 9.38%	-1.68% / 5.43%
> 15 / ≤ 15 teeth	41.35%	-6.87%	-0.93%	-1.48%	2.24%	-0.86%
≥ 8 teeth	-15.60%	-2.46%	-0.94%	-2.50%	-1.42%	-1.60%
≥ 20 teeth	-18.35%	-1.49%	-3.75%	-71.37%	23.78%	0.82%
28 teeth	7.41%					
Random Mx / Mn*						
≥ 2 teeth	0.92%	-8.46%	-0.94%	0.74%	-1.46%	-2.63%

> 15 / ≤ 15 teeth	6.60% / -29.32%	-5.04% / -28.71%	0.00% / 0.00%	0.71% / 35.38%	-2.05% / 39.06%	0.00% / 6.52%
≥ 8 teeth	0.00%	-9.92%	-1.85%	2.22%	0.00%	-1.72%
≥ 20 teeth	4.59%	-6.56%	-0.94%	5.00%	-2.84%	-1.60%
28 teeth	66.67%	-2.99%	1.25%	-28.63%	10.27%	-1.64%
Random Rt / Lt *						
≥ 2 teeth	-3.67%	-6.15%	-0.94%	-2.94%	-2.19%	-0.88%
> 15 / ≤ 15 teeth	-1.89% / -24.81%	-1.68% / -33.97%	0.00% / 0.00%	-3.57% / 18.46%	2.74% / -4.69%	0.00% / 2.17%
≥ 8 teeth	-4.59%	-6.87%	-0.93%	-0.74%	0.75%	0.00%
≥ 20 teeth	-2.75%	-4.10%	-0.94%	0.00%	2.84%	-0.80%
28 teeth	50.00%	0.00%	0.00%	-22.35%	-18.38%	-0.82%
Random Mx Rt & Mn Lt / Mx Lt & Mn Rt*						
≥ 2 teeth	-6.42%	-8.46%	-0.94%	-0.74%	-2.19%	-0.88%
> 15 / ≤ 15 teeth	-4.72% / -27.07%	-2.52% / -44.50%	0.00% / -0.96%	-1.43% / 23.08%	1.37% / 3.13%	0.00% / 2.17%
≥ 8 teeth	-7.34%	-9.92%	-0.93%	0.74%	0.75%	0.00%

≥ 20 teeth	-9.17%	-3.28%	-0.94%	2.50%	-0.71%	-0.80%
28 teeth	22.22%	0.00%	0.00%	-20.00%	-12.97%	-0.82%
CPITN (10)						
≥ 2 teeth	6.42%	-10.00%	-0.94%	-10.29%	0.73%	-4.39%
> 15 / ≤ 15 teeth	10.38% / -	0.00% / -51.20%	0.00% / -1.92%	-5.00% / 29.23%	7.53% / 14.06%	-4.20% / 2.17%
≥ 8 teeth	14.29%	-12.21%	-2.78%	-8.15%	5.22%	-6.03%
≥ 20 teeth	3.67%	-6.56%	-1.89%	3.75%	7.80%	-4.00%
28 teeth	4.59%	1.49%	-1.25%	4.71%	15.14%	-4.10%
	24.07%					
Ramfjörd (6)						
≥ 2 teeth	4.59%	-12.31%	-0.94%	1.47%	-13.87%	-1.75%
> 15 / ≤ 15 teeth	9.43% / -43.61%	-5.88% / -47.85%	1.90% / -5.77%	15.00% / -	-8.90% / -	-0.84% / 0.00%
≥ 8 teeth	3.67%	-12.98%	0.00%	29.23%	14.06%	-1.72%
≥ 20 teeth	8.26%	-9.02%	2.83%	2.96%	-8.96%	-2.40%
28 teeth	-100.00%	34.33%	7.50%	38.13%	-6.38%	-3.28%
				-100.00%	5.41%	

Mx: maxillary teeth, **Mn:** mandibular teeth, **Rt:** right side, **Lt:** left side, **Mx Rt & Mn Lt**: maxillary right and mandibular contralateral sides, **Mx Lt & Mn Rt:** maxillary left and mandibular contralateral sides, **CPITN:** community periodontal index for treatment needs.

ʊ: Percent relative bias of odds ratio of severe periodontitis on diabetes or cardiovascular disease compared to no or mild periodontitis.

§: Percent relative bias of odds ratio of moderate periodontitis on diabetes or cardiovascular disease compared to no or mild periodontitis.

***** Percent relative bias of the mean estimate of 10,000 iterations.

Appendix Table 1. Percent relative bias of the estimates of the different partial-mouth protocols for the periodontitis-diabetes (N=9,586) and the periodontitis-cardiovascular disease (N=9,556) associations using both categorical definition and mean clinical attachment loss compared to full-mouth estimates.

Full-mouth / partial-mouth protocol (number of teeth)	Exposure misclassification					
	Periodontitis - Diabetes			Periodontitis – Cardiovascular disease		
	Severe[ƨ]	Moderate[§]	Mean CAL	Severe[ƨ]	Moderate[§]	Mean CAL
Full-mouth (28)						
≥ 2 teeth	1.09	1.30	1.06	1.36	1.37	1.14
> 15 / ≤ 15 teeth	1.06 / 1.33	1.19 / 2.09	1.05 / 1.04	1.40 / 0.65	1.46 / 0.64	1.19 / 0.92
≥ 8 teeth	1.09	1.31	1.08	1.35	1.34	1.16
≥ 20 teeth	1.09	1.22	1.06	1.60	1.41	1.25
28 teeth	0.54	0.67	0.80	2.55	2.31	1.22
Mx (14)						
≥ 2 teeth	1.13	1.15	1.06	1.38	1.32	1.09
> 15 / ≤ 15 teeth	1.13 / 1.12	1.13 / 1.29	1.06 / 1.05	1.40 / 1.23	1.46 / 0.91	1.20 / 1.01
≥ 8 teeth	1.10	1.12	1.05	1.38	1.36	1.13
≥ 20 teeth	1.16	1.14	1.07	1.66	1.43	1.26
28 teeth	0.98	0.78	0.90	1.55	2.31	1.08

Mn (14)						
≥ 2 teeth	1.06	1.24	1.05	1.34	1.38	1.13
> 15 / ≤ 15 teeth	1.12 / 0.87	1.13 / 1.76	1.04 / 1.03	1.42 / 0.60	1.41 / 0.76	1.18 / 0.94
≥ 8 teeth	1.08	1.25	1.06	1.38	1.33	1.14
≥ 20 teeth	1.10	1.14	1.04	1.72	1.31	1.21
28 teeth	0.77	0.53	0.75	2.02	1.78	1.29
Rt (14)						
≥ 2 teeth	1.05	1.27	1.05	1.22	1.34	1.15
> 15 / ≤ 15 teeth	1.03 / 1.06	1.22 / 1.40	1.05 / 1.02	1.23 / 0.74	1.46 / 0.65	1.19 / 0.97
≥ 8 teeth	1.06	1.27	1.07	1.23	1.31	1.17
≥ 20 teeth	1.06	1.22	1.05	1.47	1.35	1.25
28 teeth	0.77	0.56	0.78	0.91	1.72	1.21
Lt (14)						
≥ 2 teeth	1.04	1.17	1.06	1.44	1.34	1.12
> 15 / ≤ 15 teeth	1.05 / 0.91	1.12 / 1.34	1.05 / 1.05	1.48 / 0.79	1.53 / 0.57	1.18 / 0.92
≥ 8 teeth	1.02	1.16	1.08	1.45	1.39	1.15

≥ 20 teeth	1.06	1.12	1.05	1.72	1.55	1.24
28 teeth	0.78	0.79	0.82	3.33	1.29	1.21
Mx Rt & Mn Lt (14)						
≥ 2 teeth	1.14	1.16	1.06	1.38	1.31	1.13
> 15 / ≤ 15 teeth	1.10 / 1.21	1.13 / 1.15	1.06 / 1.03	1.43 / 0.79	1.47 / 0.61	1.21 / 0.91
≥ 8 teeth	1.12	1.15	1.09	1.39	1.33	1.17
≥ 20 teeth	1.10	1.17	1.06	1.72	1.41	1.26
28 teeth	0.70	0.69	0.83	3.23	0.98	1.19
Mx Lt & Mn Rt (14)						
≥ 2 teeth	0.92	1.21	1.05	1.34	1.37	1.14
> 15 / ≤ 15 teeth	0.93 / 0.78	1.19 / 1.14	1.04 / 1.04	1.33 / 0.80	1.48 / 0.70	1.17 / 0.97
≥ 8 teeth	0.92	1.22	1.07	1.33	1.37	1.15
≥ 20 teeth	0.89	1.19	1.05	1.56	1.39	1.23
28 teeth	0.58	0.66	0.77	0.73	2.29	1.23

Random Mx / Mn*

≥ 2 teeth	1.10	1.19	1.05	1.37	1.35	1.11
	(0.92, 1.30)	(1.07, 1.33)	(1.02, 1.08)	(1.09, 1.67)	(1.16, 1.55)	(1.07, 1.15)
> 15 / ≤ 15 teeth	1.13 / 0.94	1.13 / 1.49	1.05 / 1.04	1.41 / 0.88	1.43 / 0.89	1.19 / 0.98
> 15 interval	(0.92, 1.36)	(1.01, 1.26)	(1.02, 1.08)	(1.08, 1.77)	(1.22, 1.66)	(1.14, 1.23)
≤ 15 interval	(0.67, 1.27)	(1.02, 2.08)	(0.98, 1.10)	(0.57, 1.28)	(0.57, 1.28)	(0.91, 1.05)
≥ 8 teeth	1.09	1.18	1.06	1.38	1.34	1.14
	(0.91, 1.28)	(1.06, 1.32)	(1.03, 1.09)	(1.10. 1.68)	(1.16, 1.55)	(1.09, 1.18)
≥ 20 teeth	1.14	1.14	1.05	1.68	1.37	1.23
	(0.89, 1.42)	(1.01, 1.29)	(1.02, 1.09)	(1.27, 2.15)	(1.15, 1.61)	(1.18, 1.28)
28 teeth	0.90	0.65	0.81	1.82	2.04	1.20
	(0.46, 1.50)	(0.46, 0.87)	(0.73, 0.89)	(0.79, 3.09)	(1.49, 2.67)	(1.04, 1.38)

Random Rt / Lt *						
≥ 2 teeth	1.05	1.22	1.05	1.32	1.34	1.13
	(0.90, 1.20)	(1.10, 1.33)	(1.04, 1.07)	(1.12, 1.53)	(1.19, 1.49)	(1.11, 1.15)
> 15 / ≤ 15 teeth	1.04 / 1.00	1.17 / 1.38	1.05 / 1.04	1.35 / 0.77	1.50 / 0.61	1.19 / 0.94
> 15 interval	(0.87, 1.22)	(1.06, 1.28)	(1.03, 1.07)	(1.10, 1.62)	(1.31, 1.69)	(1.16, 1.21)
≤ 15 interval	(0.77, 1.26)	(1.02, 1.81)	(1.03, 1.07)	(0.60, 0.97)	(0.46, 0.79)	(0.91, 0.98)
≥ 8 teeth	1.04	1.22	1.07	1.34	1.35	1.16
	(0.89, 1.19)	(1.10, 1.34)	(1.06, 1.09)	(1.13, 1.55)	(1.20, 1.51)	(1.14, 1.18)
≥ 20 teeth	1.06	1.17	1.05	1.60	1.45	1.24
	(0.85, 1.30)	(1.05, 1.30)	(1.03, 1.08)	(1.23, 2.00)	(1.26, 1.65)	(1.21, 1.27)
28 teeth	0.81	0.67	0.80	1.98	1.51	1.21
	(0.43, 1.32)	(0.55, 0.81)	(0.75, 0.85)	(0.57, 3.78)	(1.10, 1.97)	(1.09, 1.33)

Random Mx Rt & Mn Lt / Mx Lt & Mn Rt*						
≥ 2 teeth	1.02	1.19	1.05	1.35	1.34	1.13
	(0.88, 1.17)	(1.08, 1.30)	(1.04, 1.07)	(1.34, 1.58)	(1.20, 1.50)	(1.12, 1.15)
> 15 / ≤ 15 teeth	1.01 / 0.97	1.16 / 1.16	1.05 / 1.03	1.38 / 0.80	1.48 / 0.66	1.19 / 0.94
> 15 interval	(0.85, 1.19)	(1.05, 1.29)	(1.03, 1.07)	(1.12, 1.67)	(1.30, 1.67)	(1.17, 1.21)
≤ 15 interval	(0.75, 1.22)	(0.90, 1.46)	(1.00, 1.07)	(0.62, 1.02)	(0.52, 0.82)	(0.90, 0.98)
≥ 8 teeth	1.01	1.18	1.07	1.36	1.35	1.16
	(0.87. 1.16)	(1.07, 1.30)	(1.06, 1.09)	(1.14, 1.60)	(1.21, 1.51)	(1.14, 1.18)
≥ 20 teeth	0.99	1.18	1.05	1.64	1.40	1.24
	(0.79, 1.20)	(1.06, 1.31)	(1.03, 1.07)	(1.27, 2.05)	(1.22, 1.60)	(1.21, 1.27)

28 teeth	0.66	0.67	0.80	2.04	1.61	1.21
	(0.32, 1.10)	(0.53, 0.83)	(0.75, 0.85)	(0.63, 3.81)	(1.07, 2.25)	(1.11, 1.32)
CPITN (10)						
≥ 2 teeth	1.16	1.17	1.05	1.22	1.38	1.09
> 15 / ≤ 15 teeth	1.17 / 1.14	1.19 / 1.02	1.05 / 1.02	1.05 / 0.84	1.57 / 0.73	1.14 / 0.94
≥ 8 teeth	1.13	1.15	1.05	1.24	1.41	1.09
≥ 20 teeth	1.14	1.14	1.04	1.66	1.52	1.20
28 teeth	0.67	0.68	0.79	2.67	2.13	1.17
Ramfjörd (6)						
≥ 2 teeth	1.14	1.14	1.05	1.38	1.18	1.12
> 15 / ≤ 15 teeth	1.16 / 0.75	1.12 / 1.09	1.07 / 0.98	1.61 / 0.46	1.33 / 0.55	1.18 / 0.92
≥ 8 teeth	1.13	1.14	1.08	1.39	1.22	1.14
≥ 20 teeth	1.18	1.11	1.09	2.21	1.32	1.22
28 teeth	0.000002	0.90	0.86	0.000002	1.95	1.18

Mx: maxillary teeth, **Mn:** mandibular teeth, **Rt:** right side, **Lt:** left side, **Mx Rt & Mn Lt**: maxillary right and mandibular contralateral sides, **Mx Lt & Mn Rt:** maxillary left and mandibular contralateral sides, **CPITN:** community periodontal index for treatment needs.

ʊ: Odds ratio of severe periodontitis on diabetes or cardiovascular disease compared to no or mild periodontitis.

§: Odds ratio of moderate periodontitis on diabetes or cardiovascular disease compared to no or mild periodontitis.

***** Mean estimates and (2.5^{th}, 97.5^{th}) percentile intervals of 10,000 iterations.

Appendix Table 2. Estimates of the different partial-mouth protocols for the periodontitis-diabetes (N=9,586) and the periodontitis-cardiovascular disease (N=9,556) associations using both categorical definition and mean clinical attachment loss.

Partial-mouth protocol (number of teeth)	Outcome misclassification		
	Severe[¥]	**Moderate**[‡]	**Mean CAL***
Mx (14)			
≥ 2 teeth	4.95%	-16.00%	-20.00%
> 15 / ≤ 15 teeth	8.16% / -21.32%	-7.08% / -44.59%	50.00% / 26.67%
≥ 8 teeth	1.98%	-17.60%	-50.00%
≥ 20 teeth	8.91%	-10.08%	0.00%
28 teeth	88.46%	18.33%	-50.00%
Mn (14)			
≥ 2 teeth	-2.97%	-5.60%	-20.00%
> 15 / ≤ 15 teeth	5.10% / -38.24%	-6.19% / -17.75%	0.00% / -13.33%
≥ 8 teeth	-1.98%	-4.80%	-16.67%
≥ 20 teeth	-2.97%	-8.40%	-50.00%
28 teeth	25.00%	-18.33%	37.50%
Rt (14)			
≥ 2 teeth	-2.97%	-6.40%	-20.00%

> 15 / ≤ 15 teeth	-1.02% / -24.26%	0.00% / -37.66%	0.00% / -13.33%
≥ 8 teeth	-2.97%	-5.60%	-16.67%
≥ 20 teeth	-1.98%	-3.36%	-50.00%
28 teeth	51.92%	-16.67%	0.00%
Lt (14)			
≥ 2 teeth	-4.95%	-11.20%	20.00%
> 15 / ≤ 15 teeth	-1.02% / -33.09%	-6.19% / -38.10%	0.00% / 46.67%
≥ 8 teeth	-6.93%	-11.20%	16.67%
≥ 20 teeth	-5.94%	-9.24%	0.00%
28 teeth	26.92%	20.00%	12.50%
Mx Rt & Mn Lt (14)			
≥ 2 teeth	2.97%	-13.60%	20.00%
> 15 / ≤ 15 teeth	2.04% / -11.76%	-7.96% / -47.19%	50.00% / 6.67%
≥ 8 teeth	0.00%	-14.40%	16.67%
≥ 20 teeth	-2.97%	-7.56%	0.00%
28 teeth	9.62%	5.00%	-25.00%

Mx Lt & Mn Rt (14)			
≥ 2 teeth	-13.86%	-9.60%	0.00%
> 15 / ≤ 15 teeth	-10.20% / -43.38%	-1.77% / -48.05%	0.00% / 6.67%
≥ 8 teeth	-13.86%	-8.80%	-16.67%
≥ 20 teeth	-16.83%	-5.04%	-50.00%
28 teeth	19.23%	0.00%	12.50%
Random Mx / Mn**			
≥ 2 teeth	0.99%	-10.40%	-20.00%
> 15 / ≤ 15 teeth	7.14% / -33.82%	-6.19% / -32.90%	0.00% / 6.67%
≥ 8 teeth	0.00%	-11.20%	-33.33%
≥ 20 teeth	3.96%	-9.24%	0.00%
28 teeth	57.69%	0.00%	0.00%
Random Rt / Lt **			
≥ 2 teeth	-3.96%	-8.80%	0.00%
> 15 / ≤ 15 teeth	-1.02% / -27.94%	-3.54% / -37.23%	0.00% / 13.33%
≥ 8 teeth	-4.95%	-8.80%	0.00%

≥ 20 teeth	-3.96%	-6.72%	0.00%
28 teeth	42.31%	1.67%	0.00%
Random Mx Rt & Mn Lt / Mx Lt & Mn Rt**			
≥ 2 teeth	-6.93%	-11.20%	0.00%
> 15 / ≤ 15 teeth	-4.08% / -29.41%	-4.42% / -47.19%	0.00% / 6.67%
≥ 8 teeth	-7.92%	-12.00%	0.00%
≥ 20 teeth	-9.90%	-6.72%	0.00%
28 teeth	15.38%	3.33%	0.00%
CPITN (10)			
≥ 2 teeth	2.97%	-14.40%	-20.00%
> 15 / ≤ 15 teeth	9.18% / -20.59%	-2.65% / -55.41%	50.00% / -20.00%
≥ 8 teeth	0.99%	-15.20%	-16.67%
≥ 20 teeth	2.97%	-10.08%	0.00%
28 teeth	9.62%	1.67%	25.00%

Ramfjörd (6)			
≥ 2 teeth	6.93%	-14.40%	-20.00%
> 15 / ≤ 15 teeth	14.29% / -47.06%	-7.08% / -52.81%	100.00% / 140.00%
≥ 8 teeth	5.94%	-15.20%	0.00%
≥ 20 teeth	7.92%	-12.61%	100.00%
28 teeth	-100.00%	40.00%	-25.00%

Mx: maxillary teeth, **Mn:** mandibular teeth, **Rt:** right side, **Lt:** left side, **Mx Rt & Mn Lt**: maxillary right and mandibular contralateral sides, **Mx Lt & Mn Rt:** maxillary left and mandibular contralateral sides, **CPITN:** community periodontal index for treatment needs.

¥: Percent relative bias of odds ratio of diabetics for having severe periodontitis against no or mild periodontitis compared to non-diabetics.

‡: Percent relative bias of odds ratio of diabetics for having moderate periodontitis against no or mild periodontitis compared to non-diabetics.

***** Percent relative bias of beta-estimate of diabetics for mean clinical attachment loss compared to non-diabetics.

****** Percent relative bias of the mean estimate of 10,000 iterations.

Appendix Table 3. Percent relative bias of the estimates of the different partial-mouth protocols for the diabetes-periodontitis associations (N=9,586) using both categorical definition and mean clinical attachment loss compared to full-mouth estimates.

Full-mouth / partial-mouth protocol (number of teeth)	Outcome misclassification		
	Severe[¥]	Moderate[‡]	Mean CAL*
Full-mouth (28)			
≥ 2 teeth	1.01	1.25	0.05
> 15 / ≤ 15 teeth	0.98 / 1.36	1.13 / 2.31	0.02 / 0.15
≥ 8 teeth	1.01	1.25	0.06
≥ 20 teeth	1.01	1.19	0.02
28 teeth	0.52	0.60	-0.08
Mx (14)			
≥ 2 teeth	1.06	1.05	0.04
> 15 / ≤ 15 teeth	1.06 / 1.07	1.05 / 1.28	0.03 / 0.19
≥ 8 teeth	1.03	1.03	0.03
≥ 20 teeth	1.10	1.07	0.02
28 teeth	0.98	0.71	-0.04

Mn (14)			
≥ 2 teeth	0.98	1.18	0.04
> 15 / ≤ 15 teeth	1.03 / 0.84	1.06 / 1.90	0.02 / 0.13
≥ 8 teeth	0.99	1.19	0.05
≥ 20 teeth	0.98	1.09	0.01
28 teeth	0.65	0.49	-0.11
Rt (14)			
≥ 2 teeth	0.98	1.17	0.04
> 15 / ≤ 15 teeth	0.97 / 1.03	1.13 / 1.44	0.02 / 0.13
≥ 8 teeth	0.98	1.18	0.05
≥ 20 teeth	0.99	1.15	0.01
28 teeth	0.79	0.50	-0.08
Lt (14)			
≥ 2 teeth	0.96	1.11	0.06
> 15 / ≤ 15 teeth	0.97 / 0.91	1.06 / 1.43	0.02 / 0.22
≥ 8 teeth	0.94	1.11	0.07

≥ 20 teeth	0.95	1.08	0.02
28 teeth	0.66	0.72	-0.07
Mx Rt & Mn Lt (14)			
≥ 2 teeth	1.04	1.08	0.06
> 15 / ≤ 15 teeth	1.00 / 1.20	1.04 / 1.22	0.03 / 0.16
≥ 8 teeth	1.01	1.07	0.07
≥ 20 teeth	0.98	1.10	0.02
28 teeth	0.57	0.63	-0.06
Mx Lt & Mn Rt (14)			
≥ 2 teeth	0.87	1.13	0.05
> 15 / ≤ 15 teeth	0.88 / 0.77	1.11 / 1.20	0.02 / 0.16
≥ 8 teeth	0.87	1.14	0.05
≥ 20 teeth	0.84	1.13	0.01
28 teeth	0.62	0.60	-0.09

Random Mx / Mn**			
≥ 2 teeth	1.02	1.12	0.04
	(0.86, 1.20)	(1.00, 1.24)	(0.004, 0.08)
> 15 / ≤ 15 teeth	1.05 / 0.90	1.06 / 1.55	0.02 / 0.16
	(0.86, 1.25) / (0.63, 1.23)	(0.94, 1.18) / (1.06, 2.19)	(0.004, 0.05) / (-0.09, 0.40)
≥ 8 teeth	1.01	1.11	0.04
	(0.85, 1.18)	(0.99, 1.24)	(0.01, 0.07)
≥ 20 teeth	1.05	1.08	0.02
	(0.83, 1.30)	(0.95, 1.22)	(-0.003, 0.04)
28 teeth	0.82	0.60	-0.08
	(0.46, 1.30)	(0.41, 0.80)	(-0.11, -0.05)

Random Rt / Lt **			
≥ 2 teeth	0.97	1.14	0.05
	(0.84, 1.11)	(1.04, 1.25)	(0.03, 0.07)
> 15 / ≤ 15 teeth	0.97 / 0.98	1.09 / 1.45	0.02 / 0.17
	(0.82, 1.13) / (0.75, 1.25)	(0.99, 1.20) / (1.08, 1.93)	(0.01, 0.04) / (0.08, 0.27)
≥ 8 teeth	0.96	1.14	0.06
	(0.83, 1.10)	(1.03, 1.26)	(0.04, 0.08)
≥ 20 teeth	0.97	1.11	0.02
	(0.79, 1.18)	(1.00, 1.24)	(0.002, 0.03)
28 teeth	0.74	0.61	-0.08
	(0.44, 1.18)	(0.49, 0.73)	(-0.10, -0.06)

Random Mx Rt & Mn Lt / Mx Lt & Mn Rt**			
≥ 2 teeth	0.94 (0.82, 1.08)	1.11 (1.00, 1.21)	0.05 (0.03, 0.07)
> 15 / ≤ 15 teeth	0.94 / 0.96 (0.79, 1.10) / (0.73, 1.22)	1.08 / 1.22 (0.98, 1.19) / (0.95, 1.54)	0.02 / 0.16 (0.01, 0.04) / (0.06, 0.26)
≥ 8 teeth	0.93 (0.80, 1.07)	1.10 (1.00, 1.21)	0.06 (0.04, 0.07)
≥ 20 teeth	0.91 (0.73, 1.10)	1.11 (0.99, 1.23)	0.02 (0.003, 0.03)
28 teeth	0.60 (0.32, 0.94)	0.62 (0.49, 0.76)	-0.08 (-0.09, -0.06)

CPITN (10)			
≥ 2 teeth	1.04	1.07	0.04
> 15 / ≤ 15 teeth	1.07 / 1.08	1.10 / 1.03	0.03 / 0.12
≥ 8 teeth	1.02	1.06	0.05
≥ 20 teeth	1.04	1.07	0.02
28 teeth	0.57	0.61	-0.10
Ramfjörd (6)			
≥ 2 teeth	1.08	1.07	0.04
> 15 / ≤ 15 teeth	1.12 / 0.72	1.05 / 1.09	0.04 / -0.06
≥ 8 teeth	1.07	1.06	0.06
≥ 20 teeth	1.09	1.04	0.04
28 teeth	0.000005	0.84	-0.06

Mx: maxillary teeth, **Mn:** mandibular teeth, **Rt:** right side, **Lt:** left side, **Mx Rt & Mn Lt**: maxillary right and mandibular contralateral sides, **Mx Lt & Mn Rt:** maxillary left and mandibular contralateral sides, **CPITN:** community periodontal index for treatment needs.

¥: Odds ratio of diabetics for having severe periodontitis against no or mild periodontitis compared to non-diabetics.

‡: Odds ratio of diabetics for having moderate periodontitis against no or mild periodontitis compared to non-diabetics.

***** Beta-estimate of diabetics for mean clinical attachment loss compared to non-diabetics.

****** Mean estimates and (2.5^{th}, 97.5^{th}) percentile intervals of 10,000 iterations.

Appendix Table 4. Estimates of the different partial-mouth protocols for the diabetes-periodontitis associations (N=9,586) using both categorical definition and mean clinical attachment loss.

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