Relationship between Peri-implantitis and Cardiovascular Diseases

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ABSTRACT

Objectives: The purpose of this study was to assess the relationship between peri-implantitis and cardiovascular diseases.

Methods: A case-control design, cross-sectional study was conducted to evaluate the prevalence of peri-implantitis among individuals diagnosed with cardiovascular diseases (CVD). Health and CVD history were obtained through structured questionnaire. Participants who had at least one implant in function for more than 6 months were recruited. Individuals without CVD were grouped as "Control', and participants in the "Case" CVD group were recruited only when the dental implants were placed prior to CVD diagnosis. Among the "Case" group, individuals with peri-implantitis were included in the final analysis only when the per-implantitis onset was evidenced radiographically prior to the diagnosis of the CVD. Clinical and radiographic examinations were performed; samples of serum, peri-implant crevicular fluid (PICF), and gingival crevicular fluid (GCF) at the most severe diseased sites were collected to evaluate the pro-inflammatory cytokine profile. Subgingival plaque from the peri-implantitis sulci were analyzed using 16S rRNA next-generation sequencing and real-time qPCR technologies.

Results: A higher prevalence of peri-implantitis (with detectable radiographic bone loss) in the CVD group (OR= 1.48, 95% CI= 0.71 to 3.11, p= 0.30) was found. Furthermore, moderate to severe peri-implantitis (radiographic bone loss ≥ 2mm) was significantly associated with cardiovascular diseases (OR= 2.18, 95% CI= 1.02 to 4.67, p=0.04), but was no longer observed after controlling for multiple significant confounding factors. The microbial community among the CVD group and peri-implantitis group demonstrated a more divergent profile compared to the control and healthy implant group. Predominantly anaerobic microorganisms at the peri-implantitis niche were associated with deeper pockets and severe bone loss. A higher bacterial counts (gene copies) of *Prophylomonas gingivalis (P. gingivalis)* was observed in the CVD group. Secondly, peri-implantitis pockets appeared to harbor higher *Fusobacterium nucleatum* (*F. nucleatum) DNA* in a dose-responsive relationship with the severity of peri-implant disease. *Tannerella forsythia (T. forsythia)* was significantly higher only in the severe peri-implantitis (radiographic bone loss> 4 mm). Serum fibrinogen was significantly higher in the CVD group and moderate to severe peri-implantitis (radiographic bone loss≥ 2mm) sites when compared to the control or healthy implant groups. A similar trend was observed in the serum interleukin (IL)-6, tumor necrosis factor (TNF-α), and osteoprotegerin (OPG). PICF TNF-α was predominantly higher in the CVD group. This coincided with the local peri-implant inflammation. Matrix metalloproteinase (MMP)-8, IL-1β, and tissue inhibited metalloproteinase (TIMP)-2 displayed fair accuracy in predicting peri-implant disease. The sensitivity increased when combined with the bacterial concentration of *T. forsythia* and *F. nucleatum*. Finally, OPG appeared to be the only GCF biomarker correlated with peri-implant disease.

Conclusions: Moderate to severe peri-implantitis (radiographic bone loss ≥ 2mm) was observed to be a mild associative positive risk factor for the development of cardiovascular disease. However, a significant association was not observed after multivariable adjustment. Low-grade chronic inflammation around diseased dental implants, especially when the disease severity and tissue destruction was increased, may be linked to atherosclerosis and cardiovascular disease by increasing the overall inflammation burden on patients at risk for CVD.

Keywords: Dental implants, Peri-implantitis, Cardiovascular disease, Systemic inflammation, Plaque Biofilm, Microbiology, Protein cytokine

Dedication

I would like to dedicate this thesis to all who believed in me and supported me throughout my residency. My parents, Cheng-Hsiung Wang and Shu-Jen Chien, who supported me with all of their love and showed me the resilience through their examples. Thank you for being the strongest beacon in my heart and giving me the courage and strength in this long journey to realize my dream. I want to thank my partner, Yi-Chien Tung, who supported me in the highs-and-lows every day throughout my residency. This thesis wouldn't be made possible without her support during the hardest times. My younger brothers and sister, Jen-Chieh Wang, Jen-Yi Wang, and Yi-Hsuan Wang, deserve my wholehearted thanks for all the support and encouragement in every step along the way. They are the greatest blessing in my life. Special love to my three nephews that give me the tremendous joy and hope to finish this residency.

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Chapters

Abbreviations

AAP	American Academy of Periodontology
ACC	American College of Cardiology
AF	atrial fibrillation
AHA	American Heart Association
AP	angina pectoris
ASCVD	atherosclerotic cardiovascular disease
BIC	bone-to-implant contact
BMI	body mass index

BOP	bleeding on probing
BP	blood pressure
CAD	coronary artery disease
CAL	clinical attachment loss
CDC	Centers for Disease Control and Prevention
CHD	coronary heart disease
CHF	congestive heart failure
CI	confidence interval
CVD	cardiovascular disease
DBP	diastolic blood pressure
DALY	disability-adjusted life-years
DM	diabetes mellitus
EFP	European Federation of Periodontology
FPG	fasting plasma glucose
GBD	Global Burden of Disease
HA	hydroxyapatite
HBP	high blood pressure
HD	heart disease
HDL-C	high-density lipoprotein cholesterol
hsCRP	high-sensitivity C-reactive protein
HTN	hypertension
IHD	ischemic heart disease
IR	insulin resistance
IRB	Institutional Review Board
KMW	keratinized mucosa width
LDL-C	low-density lipoprotein cholesterol
MetS	metabolic syndrome
MHO	metabolically healthy obesity
MI	myocardial infarction
MU	Peri-implant mucositis
NHANES	National Health and Nutrition Examination Survey
NHLBI	National Heart, Lung, and Blood Institute
OR	odds ratio
OTU	operational taxonomic unit
PAD	peripheral artery disease
PI	peri-implantitis
RA	rheumatoid arthritis
RT-qPCR	real-time quantitative polymerase chain reaction
REC	recession
SBP	systolic blood pressure
SDI	Socio-demographic Index
SD	standard deviation
SLA	sandblasted and acid-Etched
TC	total cholesterol
VTE	venous thromboembolism
WHF	World Heart Federation

Chapter I: Introduction

Peri-implantitis is a plaque-associated pathological condition occurring in tissue around dental implants characterized by inflammation in the peri-implant mucosa and subsequent progressive loss of supporting bone (Berglundh et al. 2018). Similarly, peri-implant mucositis presents with soft tissue inflammation in the absence of supporting bone loss or continuing marginal bone loss after initial bone remodeling or treatment (Heitz-

Mayfield and Salvi 2018). Collectively, it can be referred to as peri-implant disease, which are the biological complications surrounding dental implants and their restorative components induced by the accumulation of a bacterial biofilm (Renvert et al. 2018). Conversely, peri-implant health is defined as intact hard and soft tissue compartments free of inflammation (Araujo and Lindhe 2018). The prevalence of peri-implantitis largely varied from the different cut-off threshold of bone loss (Schwarz et al. 2018). In a recent systematic review, the prevalence of peri-implant mucositis and peri-implantitis were estimated to range from 19% to 65% and from 1% to 47% with a negative relationship with the threshold for bone loss (Derks and Tomasi 2015).

Cardiovascular diseases (CVD) are the leading cause among non-communicable diseases for global motality rates and accounts for one-third of total mortality (World Health Organization 2014). On the basis of NHANES 2013 to 2016 data, prevalence of CVD (comprising coronary heart disease, heart failure, stroke, and hypertension) in adults ≥ 20 years of age is 48.0% overall (121.5 million in 2016) and increases with age in both genders. CVD prevalence excluding hypertension is 9.0% overall (Centers for Disease Control and Prevention 2019). CVD has been linked to chronic periodontitis based on a significant body of epidemiologic evidence. Biologic plausibility related to periodontal bacteremia and mounting systemic inflammatory burden has been widely investigated (Dietrich et al. 2013; Sanz et al. 2020). Similar to chronic periodontitis, the chronic inflammation around dental implants was found consistently harboring periopathogenic bacteria (de Waal et al. 2017; Schwarz et al. 2018) and augmented immune response via the accumulating evidence of increased pro-inflammatory cytokines at peri-implantitis sites (Duarte et al. 2016; Gürlek et al. 2017). On the basis of similarities in microbial profiles and the elevated chronic inflammatory response between periodontitis and peri-implantitis, a potential link between peri-implantitis and cardiovascular disease was suspected. It has been reported that there was a higher prevalence of CVD among individuals with peri-implantitis (Renvert et al. 2014; Dalago et al. 2017). The prevalence of CVD was statistically significantly associated with one of the periodontal pathogens, *Prevotella intermedia (P. intermedia),* at the peri-implantitis sites (Lachmann et al. 2013). It was proposed that the chronic inflammation associated with peri-implant disease may induce and escalate systemic low-grade inflammation and increase the risk of cardiovascular disease via a similar infectious axis between periodontitis and CVD. The purpose of this study was to assess the relationship between peri-implantitis and cardiovascular diseases (CVD).

Chapter II: Background of cardiovascular and peri-implant diseases

A. Disease Burden of Cardiovascular Disease

The highest global burden of non-communicable disease (NCDs) arises from cardiovascular diseases (CVD), which are responsible for 17.9 million deaths (a third of total mortality) and 45% of NCD-induced mortality (Roth et al. 2017). Ischemic heart disease, stroke, hypertensive heart disease, rheumatic heart disease, cardiomyopathy and arterial fibrillation cause over 95% of CVD-related deaths (Roth et al. 2015). Cardiovascular diseases (CVD) are the leading cause of death in the United States. Heart disease and stroke were the first and fourth in the ranking of the 10 leading causes of death (Murphy et al. 2018). It was also the most expensive chronic condition generating not only overall medical expenditures but also the loss of workplace productivity estimated at $207 billion each year in the United States (Goetzel et al. 2017). The direct healthcare costs of CVD are projected to triple from $273 billion in 2010 to $818 billion in 2030; and the indirect costs associated with lost productivity was projected to increase from $172 billion to $276 billion (Fonarow et al. 2015). According to a recent systematic metric measuring the age-related morbidity and mortality at population level, using the data from the Global Burden of Diseases, Injuries, and Risk Factors Study, 92 diseases were identified as age related, accounting for 51.3% of all global burden among adults in 2017(Chang et al. 2019). CVD is considered the most important age-related disease, which was defined as a disease with incidence rate increasing quadratically with age, and measured with their age-related burden, namely the sum of disability-adjusted life-years (DALYs). The rate of age-related burden ranged from 137.8 DALYs per 100 adults in high socio-demographic index (SDI) countries to 265.9 DALYs in low SDI countries. CVD accounted for 38.4% of the age-related disease burden; and ischemic heart disease, intracerebral hemorrhage (stroke) were the two leading age-related diseases with most deaths and DALYs globally (Kyu et al. 2018).

B. Prevalence of Cardiovascular Disease

Annually, the American Heart Association (AHA) in conjunction with the National Institutes of Health (NIH) publishes current statistics related to heart disease, stroke, and the cardiovascular risk factors (Virani et al. 2020). This report presents the latest updates on a range of major clinical heart and circulatory disease

conditions and is collectively termed the "Total Cardiovascular Disease (CVD)" including stroke, congenital heart disease, rhythm disorders, subclinical atherosclerosis, coronary heart disease, heart failure, valvular disease, venous disease, and peripheral artery disease. The AHA statistics updated in 2020 first focused on the basis of NHANES 2013-2016 data. The prevalence of the Total CVD (Comprising CHD, HF, stroke and hypertension) in adults (≥ 20 years of age) is 48% (Virani et al. 2020). CVD prevalence excluding hypertension (coronary heart disease (CHD), heart failure (HF), and stroke only) is 9% overall (National Center for Health Statistics 2017). More recently, the 2017 National Health Interview Survey showed the age-adjusted prevalence of all types of heart disease (HD) was 10.6%; and the age-adjusted prevalence of HD, coronary artery disease (CAD), hypertension, and stroke was higher in men (11.8%, 7.2%, 26%, and 3.3%, respectively) than women (9.5%, 4.2%, 23.1%, and 2.5%, respectively)(Blackwell and Villarroel 2018).

C. Risk factors for Cardiovascular Disease

The CVD-associated risk factors have been prodigiously investigated for decades. A recent study calculated the Global Burden of Disease (GBD) among US adults and found that CVD is attributable to dietary risks, high blood pressure (HBP), high Body Mass Index (BMI), high total cholesterol (TC) level, high fasting plasma glucose (FPG) level, tobacco smoking, and low levels of physical activity(in decreasing order of contribution)(Virani et al. 2020). Unfortunately, 99% of the US adult population has at least one of the seven cardiovascular health risks. The combined contribution of these risk factors escalated medical spending by 213.6% per person per year(Goetzel et al. 2017). The prevalence of uncontrolled risk factors for CVD among adults in US was estimated at 47% with 1 of the 3 well-established key risk factors(HBP, high cholesterol, and smoking) (Fryar et al. 2012). Another study identified HBP as the most important single preventable risk factor for cardiovascular mortality in US and was responsible for 45% of all cardiovascular deaths. Additional risk factors for cardiovascular mortality were insulin resistance, overweight/obesity, low physical activity, high low-density lipoprotein cholesterol (LDL-C), smoking, high dietary salt and transfatty acids, and low dietary omega-3 fatty acids (Danaei et al. 2009). Other than traditional CVD risk factors, chronic kidney disease (CKD), systolic blood pressure (SBP) variability, migraine, severe mental illness, systemic lupus erythematous, use of corticosteroid or antipsychotic medications, early-age menopause, HIV, type 1 diabetes mellitus (DM), socioeconomic deprivation, and erectile dysfunction were reported as non-traditional CVD risk factors (Hippisley-Cox et al. 2017; Virani et al. 2020).

In recent years, the AHA has defined cardiovascular health based on 7 risk factors-Life' Simple 7 (American Heart Association), which include key health behaviors (smoking, physical activity, diet, and weight) and core health factors (cholesterol, blood pressure and glucose control). People with at least five factors fulfilling the ideal Life's Simple 7 metric had reduced risk for heart-related death by 78% compared to people did not meet the ideal metric (Ford et al. 2012). The major risk factors other than the health behaviors included obesity, high blood cholesterol and other lipids, high blood pressure, DM, and metabolic syndrome (MetS); which also constitute the common comorbidities of CVD.

Obesity is a major risk factors for CVD, including CHD (Klein et al. 2004), stroke(Poirier et al. 2009), atrial fibrillation (AF)(Mi et al. 2016; Aune et al. 2017), venous thromboembolism (VTE)(Mi et al. 2016), and congestive heart failure (CHF)(Virani et al. 2020). According to a systematic analysis based on The Global Burden of Disease Study in 2017, obesity remained a key factor driving the leading causes of burden in high-middle and middle SDI countries (e.g., ischemic heart disease, ischemic stroke, diabetes, low back and neck pain)(Kyu et al. 2018). For adults, BMI was categorized as overweight ($25.0 \leq BMI \geq 29.9$ kg/m^2) and obese class I (BMI 30-35 kg/m^2), class II (BMI >35-39.9 kg/m^2), and class III (BMI $\geq$ 40 kg/m^2) (National Heart, Lung, and Blood Institute). According to 2015-2016 data from NHANES, the prevalence of obesity among adults was 38.3% (36.0% of males and 40.4% of females), including 7.7% with class III (severe) obesity or a BMI$\geq$ 40 kg/m^2 (5.4% of males and 9.8% of females)(Hales et al. 2018). In a population-based longitudinal study, approximately half of the older adults (45-85 year of age) with metabolically healthy obesity (MHO) developed MetS and exhibited an increased risk for CVD compared to those with stable metabolically healthy obesity or healthy normal weight. The authors concluded that MHO is not a low-risk state for cardiovascular disease (Mongraw-Chaffin et al. 2018). This finding is in line with a recent meta-analysis demonstrating that CVD risk was higher in MHO than metabolically healthy normal-weight participants (RR=1.45), but still lower than the individuals were metabolically unhealthy normal-weight (RR=2.07) and obese (RR=2.31) participants (Eckel et al. 2016). Investigators often define metabolically healthy obesity by the absence of MetS, the absence of insulin resistance (IR), or less often, the absence of abdominal adiposity or higher cardiorespiratory fitness(Stefan et al. 2013). A more strict criteria with simultaneous absence of high BP, dyslipidemia and hyperglycemia may be suggested for identification of a benign obese phenotype(Eckel et al. 2016).

Cholesterol is a primary risk factor for the development of atherosclerotic cardiovascular disease (ASCVD). Hypercholesterolemia (high LDL-C) remained the fifth-leading risk factor for mortality from 1990 to 2017(GBD Risk Factor Collaborators 2018). In adults, a total cholesterol (TC) of 200 to 239 mg/dL, measured by low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol and triglycerides, is considered borderline high and ≥ 240 mg/dL is considered high. According to an unpublished NHANES data from 2013 to 2016 (National Center for Health Statistics 2017), among adults ≥ 20 years of age, the mean TC level was 190.08 mg/dL. The prevalence of TC ≥200 mg/dL was 38.2% including hypercholesterolemia (11.7%). Other lipid subfractions including LDL cholesterol (LDL-C) was 112.1 mg/dL on average among adults, and the age-adjusted prevalence of high LDL-C (≥ 130 mg/dL) was 29.4%. HDL cholesterol (HDL-C) was 54.2 mg/dL and the age-adjusted prevalence for HDL-C < 40 mg/dL was 28.5% in males and 8.9% in females. Lastly, the mean triglyceride was 95.6 mg/dL, and approximately 22.2% of adults had high triglyceride levels (≥ 150 mg/dL). Subclinical atherosclerosis (plaque or coronary artery calcification) was recognized among half (49.7%) of individuals without other cardiovascular risk factors and was associated with LDL-C at levels currently considered normal. A linear and significant increase was found in the prevalence of atherosclerosis from LDL-C 60-70 mg/dL category to the 150-160 category (from 11% to 64%) (Fernández-Friera et al. 2017). Long-term exposure to elevated cholesterol levels (non-HDL-C ≥ 160 mg/dL) can lead to coronary artery disease (CAD) in later life with an adjusted HR, 1.39 per decade of hyperlipidemia (Navar-Boggan et al. 2015). Particularly, among these makers, LDL-C is the dominant form of atherogenic cholesterol. Additionally, very low-density lipoprotein (VLDL) as the chief carrier of triglycerides also proved to be significantly atherogenic(Grundy et al. 2019).

High blood pressure (HBP) was ubiquitously recognized as the major risk factor for CVD and stroke (Chobanian 2003; Virani et al. 2020). The AHA has identified BP <120/<80 mmHg for adults as 1 of the 7 components of ideal cardiovascular health. This represented for 55.1% in adults (20-49 years of age) and 19.9% of adults ≥50 years of age(Lloyd-Jones 2010). HBP was defined in the 2017 Hypertension Clinical Practice Guidelines as systolic blood pressure (SBP) ≥130 mmHg or diastolic blood pressure (DBP) ≥ 80 mmHg; or self -reported antihypertensive medicine use (Whelton et al. 2018). The prevalence of HBP among US adults was 45.6% using 2017 guideline threshold; and the prevalence increased with age (26.1% vs. 59.2% vs 78.2 among those 20-44 vs. 45-64 vs. ≥65 years of age) (Virani et al. 2020). Male predominance was noted among adults up to 64 years of age; but females were higher than males for those ≥ 65 years of age (National Center for Health

Statistics 2017). Genetic or family history has been associated with an increased risk of CVD and myocardial infarction (MI). Gene-environment interactions are considered important in the pathophysiology of hypertension (Cooper-DeHoff and Johnson 2016). The largest lifetime risk for CVD existed in angina, MI, and stroke. In a study of 1.25 million adults with hypertension.at age 60, the prevalence was 60.2% for those with hypertension compared with 44.6% among those without hypertension.

Diabetes mellitus (DM) is characterized by glucose dysregulation. It is diagnosed based on a fasting plasma glucose (FPG) ≥ 126 mg/dL, 2h post-challenge glucose ≥ 200 mg/dL during oral glucose tolerance test, random glucose ≥ 200 mg/dL with symptoms of hyperglycemia, or HbA1C ≥ 6.5%(American Diabetes Association 2010). Type 2 DM, which accounts for 90-95% of DM, is a major risk factor for CVD, including CHD and stroke (Emerging Risk Factors Collaboration 2010). The prevalence of diagnosed DM was 10% among US adults based on the data from NHANES 2013 to 2016 (3.7% of undiagnosed DM and 37.6% of prediabetes) (National Center for Health Statistics 2017). Type 2 DM and insulin resistance was reportedly associated with increased risk for CVD mortality (Liu et al. 2016b). The altered glucose metabolism correlated strongly with diet, contributed to obesity and type 2 DM (Joseph et al. 2017), and accounted for 90% of the burden of stroke (O'Donnell et al. 2016).

The proposed mechanisms of the association is poorly understood, but may be related to oxidative stress, low-grade inflammation, and endothelial dysfunction subsequent to hyperglycemia (Matheus et al. 2013). More importantly, it could be explained by the multiple shared risk factors between DM and CVD, including hypertension, dyslipidemia, obesity, smoking, and lack of physical activity (Leon and Maddox 2015).

Metabolic syndrome(MetS) is a multicomponent risk factor for CVD and type 2 DM reflecting a cluster of cardiometabolic risk factors related to abdominal obesity and insulin resistance(Virani et al. 2020). It is based on the presence of any 3 of the following 5 risk factors, including FPG ≥ 100 mg/dL, HDL-C <40 mg/dL, triglycerides ≥ 150 mf/dL, SBP ≥ 130 mmHg or DBP ≥ 85 mmHg (or undergoing drug treatment for those conditions), and waist circumference >102 cm in males (>88 cm in females) (Alberti et al. 2009). The overall prevalence of MetS was 34.3% and increased with age (from 19.3% among 20-39 years of age to 54.9 among those ≥ 60 years of age(Shin et al. 2018). Obesity, parental history of DM, childhood MetS, BMI, and heart rate were risk factors for MetS(Virani et al. 2020). MetS was correlated with MI, stroke, CVD morbidity and all-cause mortality(Mottillo et al. 2010).

D. The relationship between CVD and chronic oral infection

Chronic oral infections (dental caries, periodontal diseases including gingivitis and periodontitis) are among the most common chronic inflammatory diseases worldwide (Petersen et al. 2005). Periodontitis, being the sixth most common human disease, affects 11.2% of the world's population (Kaushal et al. 2019). Oral diseases (mainly periodontitis and caries) contributed the most to the "years lost to disability (YLD)" in age-standardized prevalence rates from 354 diseases globally (Forouzanfar et al. 2016). Growing evidence supports the independent association between severe periodontitis and several chronic non-communicable diseases (NCDs), including diabetes (Chapple et al. 2013; Sanz et al. 2018), cardiovascular disease (Tonetti and Van Dyke 2013; Sanz et al. 2020), chronic obstructive pulmonary disease (Linden et al. 2013), and chronic kidney disease(Sharma et al. 2016). Severe periodontitis appears to be a modifiable non-traditional risk factor for CVD, significantly associated with all-cause and cardiovascular mortality (Linden et al. 2012), particularly among the populations with multi-morbidity (Sharma et al. 2016; Liccardo et al. 2019).

The term "cardiovascular disease" (CVD) was largely used as a general term for an atherosclerotic diseases, principally coronary heart disease, cerebrovascular disease and peripheral vascular disease. The Joint Consensus Report published by the World Heart Federation (WHF) and European Federation of Periodontology (EFP) in 2019 (Sanz et al. 2020) examined the epidemiologic evidence on the association between periodontitis and CVD following the report of the joint workshop between EFP and the American Academy of Periodontology in 2013 (Dietrich et al. 2013). The epidemiological evidence belongs to a variety of CVD categories: (1) subclinical cardiovascular disease (endothelial dysfunction, measured by mean carotid intima-media thickness) (de Boer et al. 2014) (2) coronary heart disease (myocardial infarction and other coronary events) (Renvert et al. 2010; Yu et al. 2015; Rydén et al. 2016; Herrera et al. 2020) (3) cerebrovascular disease or stroke (Sen et al. 2018; Aarabi et al. 2019) (4) peripheral artery disease (Ahn et al. 2016; Yang et al. 2018)(5) other CVD or conditions (heart failure, atrial fibrillation) (Chen et al. 2016) and (6) secondary(recurrent) cardiovascular events (Reichert et al. 2016). To answer the first question: "Is periodontitis a risk factor/indicator for the CVD?", evidence mainly centers on coronary heart disease and cerebrovascular disease (Leira et al. 2017; Sanz et al. 2020). To answer the reverse question "Do people with a history of CVD have a different incidence or progression of

periodontitis?" the consensus report concluded that there's currently limited evidence that CVD is a risk factor for the onset or progression of periodontitis (Sanz et al. 2020).

The direct cause of CVD appears to be the rupture and thrombosis of atherosclerotic plaques resulting in blood flow inhibition and thus induction of CVD (Frostegård 2013). Atherosclerotic vascular disease is the leading cause of MI and cerebrovascular event and is now considered a chronic inflammatory disease (Libby et al. 2009). Chronic inflammation plays a pivotal role in the initiation and progression of atherosclerosis and the immune response, involving multiple cell types, including macrophages, T-lymphocytes, mast cells, endothelial and smooth muscle cells (Conti and Shaik-Dasthagirisaeb 2015). Other than traditional risk factors, it has been hypothesized that various stimuli including chronic infection can trigger and sustain the state of heightened inflammation, eventually leading to the damage of vascular endothelium (Pothineni et al. 2017). Several biologically plausible mechanisms of the impact of periodontitis on CVD have been proposed over the past decades. Currently, the evidence has been centered on the translocated circulating oral microbiota directly or indirectly inducing systemic inflammation impacting atherothrombogenesis (Dietrich et al. 2013). Periodontal bacteria have been identified distantly in atherosclerotic plaque (Kozarov et al. 2005; Ott et al. 2006; Fernandes et al. 2014; Figuero et al. 2014). In an animal model, infection with *Porphyromonas gingivalis* (*P. gingivalis*) increases atherosclerotic plaque volume with the accumulation of cholesterol esters and inflammatory mediators (Hayashi et al. 2011). Human serum IgA antibodies to *P. gingivalis* and other periodontal bacteria was found higher in patients with CVD and ischemic stroke than in controls (Pussinen et al. 2004; Hosomi et al. 2012; Aoyama et al. 2018). Secondly, periodontitis causes a local and systemic inflammatory response with increased counts of leukocytes, C-reactive protein (CRP), fibrinogen, cell adhesion molecules, and pro-inflammatory cytokines (Mustapha et al. 2007). Inflammatory response to the putative periodontal bacterial challenges (lipopolysaccharides) produce inflammatory mediators, including TNF-α, prostaglandin, interleukin (IL) and proteolytic enzymes like matrix metalloproteinases (MMP), etc. (Herrera et al. 2020). In addition, dyslipidemia was found to be associated with periodontitis (Thapa and Wei 2016; Lee et al. 2018), and recent data suggests a synergistic effect of both periodontal bacteria and lipid-induced inflammation in the pathogenesis of CVD (Køllgaard et al. 2017). This might be explained by the increased reactive oxygen inducing structural modifications in circulating lipoproteins (Lönn et al. 2018) and altered gene-encoding cholesterol metabolism (Liu et al. 2016a).

With the increased prevalence of dental implant therapy, chronic inflammation around dental implants is an emerging global burden among oral conditions. Peri-implant diseases are considered biological complications around dental implants induced by the accumulation of bacterial biofilm (Lang et al. 2011; Sanz et al. 2012; Renvert et al. 2018). It includes peri-implant mucositis, soft tissue inflammation in the absence of bone loss (Heitz-Mayfield and Salvi 2018) and peri-implantitis, characterized by the inflammation within the peri-implant mucosa and progressive bone loss (Berglundh et al. 2018). The state of peri-implant health was defined as healthy peri-implant mucosa and alveolar bone without signs of inflammation (free of profuse bleeding upon probing or suppuration) (Araujo and Lindhe 2018). The prevalence was greatly influenced by the cut-off threshold of disease definition. In the past decade, a cut-off level of 2 mm was adopted in the majority of published literature (Sanz et al. 2012; Schwarz et al. 2018). The prevalence has been reported as 45% with a detectable bone loss > 0.5 mm on the patient level. If the cut-off threshold of bone loss was changed to ≥ 2 mm, which was defined as moderate to severe peri-implantitis, the prevalence was diagnosed as 14.5% (Derks et al. 2016). Similar range of variability in prevalence on subject level (11.3% to 47.1%) was reported with different cut-off threshold from detectable (incipient) to bone loss > 3mm (Koldsland et al. 2010). In a systematic review, the prevalence of peri-implant mucositis and peri-implantitis were estimated ranging from 19% to 65% (mean 43%) and from 1% to 47% (mean 22%) with a negative relationship with the threshold for bone loss (Derks and Tomasi 2015). The 2017 World Workshop defined peri-implantitis as progressive bone loss at 1-year following the delivery of the implant-supported prosthesis. In the absence of initial radiographs and peri-implant pocket depths (PPD), radiographic evidence of bone level ≥ 3 mm and/or PPD ≥ 6 mm in conjunction with profuse bleeding was required for the diagnosis of peri-implantitis (Renvert et al. 2018).

Peri-implantitis occurs primarily as a result of an overwhelming bacteria insult and subsequent host immune response (Rosen et al. 2013). Peri-implant mucositis is assumed to precede peri-implantitis mirroring the progression of gingivitis to periodontitis (Jepsen et al. 2015). Inflammation of the peri-implant mucosa is dominated by plasma cells and lymphocytes (Sanz et al. 1991; Cornelini et al. 2001; Bullon et al. 2004) with larger inflammatory infiltrates with denser vascular structure outside the lesion (Carcuac and Berglundh 2014) and larger proportions of polymorphonuclear leukocytes (PMN) and macrophages (Berglundh et al. 2004). Peri-implantitis has been shown to be a polymicrobial infection around dental implants and their related prosthetic components (Shibli et al. 2008; Persson et al. 2010; Hallström et al. 2012). Results from animal and human cross-sectional studies demonstrated that similar bacterial species, mainly Gram negative anaerobes, such as *P.*

gingivalis, *Prevotella intermedia* (*P. intermedia*), *Tannerella forsythia* (*T. forsythia*), and *Fusobacterium nucleatum* (*F. nucleatum*) were found in both periodontitis and peri-implantitis (De Boever and De Boever 2006; Heitz-Mayfield and Lang 2010; Mombelli and Décaillet 2011; Persson and Renvert 2014; de Waal et al. 2017; Gürlek et al. 2017; Schwarz et al. 2018). Moreover, observational studies had linked peri-implantitis with opportunistic pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* (*S. aureus*) (Leonhardt et al. 1999; Mombelli and Décaillet 2011), fungal organisms (Schwarz et al. 2015), and viruses (Jankovic et al. 2011). Compared to periodontitis, microbial communities of peri-implantitis were more complex and heterogenous (Padial-Molina et al. 2016; Rakic et al. 2016). Koyanagi and his colleagues used 16S rRNA sequencing to identify the phylogenetic tree of bacterial species in peri-implantitis and periodontitis. The microbial composition of peri-implantitis was more diverse than that of periodontitis. *Fusobacterium spp.* and *Streptococcus spp.* were predominant in both, while bacteria such as *Parvimonas micra* were only detected in sites with peri-implantitis (Koyanagi et al. 2013). Belibasakis et al. had identified that *Spirochetes* of the *Treponema* and *Synergistetes* cluster A are highly associated with peri-implantitis (Belibasakis et al. 2016). There were contradictory observations of opportunistic *S. aureus* in the peri-implant lesion. It once appeared to play a predominant role for the development of a peri-implantitis (Renvert et al. 2007; Renvert et al. 2008; Salvi et al. 2008; Zhuang et al. 2016), especially associated with suppurative implant sites (Leonhardt et al. 1999; Kronström et al. 2001; Albertini et al. 2015); Revert et al. found that after treatment of most severe peri-implantitis, increasing levels of IL-1β and *S. aureus* (r^2=0.9) were found only at implants in a non-stable condition (Renvert et al. 2017). However, conflicting results had been reported (Canullo et al. 2015; de Waal et al. 2017). Nevertheless, *S. aureus* was consistently found to be a frequent isolate pathogen in the oral cavity, which has been seen as an important source in terms of cross-infection and dissemination to other body sites (McCormack et al. 2015). More importantly, *Staphylococcus aureus* bacteremia (SAB) was significantly associated with severe cardiovascular consequences, including chronic heart failure (Smit et al. 2016), infective endocarditis (Fowler et al. 2005; Salgado-Pabón et al. 2013; Baddour et al. 2015), pericardial infection after cardiac surgery (Chamis et al. 2001; Mozaffari et al. 2014), and cardiovascular implantable electronic device (CIED) infection(Sohail et al. 2015; Maskarinec et al. 2017). As a result, it has raised interest among medical and dental professionals.

A cross-sectional study has proved a similar pattern of host response and bone destruction between peri-implantitis and periodontitis. There was a significant increase in IL-1β, RANKL levels in peri-implantitis and

periodontitis/ gingivitis sites compared to healthy implants. (Gürlek et al. 2017). The immune response at peri-implantitis sites was similarly high (or higher) compared to periodontitis and can also be observed among different individuals (Ata-Ali et al. 2015; Recker et al. 2015; Cionca et al. 2016). Recent systematic reviews demonstrated pro-inflammatory cytokines, mostly IL-1β and TNF-α were significantly increased at mucositis and peri-implantitis sites (Faot et al. 2015; Duarte et al. 2016; Ghassib et al. 2019). Given the similarities of microbial profiles and the heightened immune response towards chronic inflammation, a potential link between peri-implantitis and CVD was proposed. A recent study has shown that patients with peri-implantitis had a high prevalence of moderate plaque and bleeding upon probing (BOP), and a significant co-morbidity occurred in individuals with cardiovascular diseases (27%). CVD was statistically significantly associated with the prevalence and concentration of *P. intermedia* in the peri-implantitis sulcus (Lachmann et al. 2013). In a retrospective study investigating the risk factors related to peri-implantitis, a history of CVD was found in 27.3% of individuals with peri-implantitis, and the odds ratio (OR) of having peri-implantitis and a history of CVD was 8.7 (95% CI: 1.9, 40.3 $p<0.006$)(Renvert et al. 2014). It was also observed in a cross-sectional study that the heart disorder associated with peri-implantitis with a significant difference in prevalence (13.1% vs. 6.9%, $p=0.012$)(Dalago et al. 2017). On the other hand, systemic low-grade inflammation has been associated with coronary heart disease (Koenig 2018). The association could be attributed to an inflammatory marker such as C reactive protein or a bacteria potentially linked to the chronic infection, such as *Chlamydia pneumoniae* (Danesh et al. 2000), or chronic gastric infection with *Helicobacter pylori*(Danesh et al. 1997). Classic vascular risk factors (such as smoking, obesity, insulin resistance, and metabolic syndrome) were also considered(Lopez-Candales et al. 2017). The inflammation associated with peri-implant mucositis and peri-implantitis and their potential to induce systemic low-grade inflammation has recently garnered attention in the research community (Golub and Lee 2020; Sorsa et al. 2020). The challenge of implant dentistry is no longer just placing a titanium implant in bone, patients receiving implants often presented with advanced age, function dependency, systemic comorbid conditions, and frailty. Naturally, aging leads to deterioration of the host defense towards bacterial challenges and adverse impact on healing capacity and peri-implant health (Ebersole et al. 2018; Schimmel et al. 2018). It has been widely conceptualized that chronic antigenic stress may mount an inflammatory response which was associated with the pathogenesis of all age-related diseases. Thus, underlying local low-grade inflammatory activity around dental implants triggered by asymptomatic infection with bacterium, may lead to increased levels of circulating inflammatory mediators such as TNF-α, IL-6, IL-2 receptor, CRP, and

cholesterol (Bruunsgaard et al. 2003; Krabbe et al. 2004); particularly in the aging population or people with multiple comorbid diseases(Lopez-Candales et al. 2017).

In contrast to the above, it was reported that patients with CVD, including ischemic heart disease, stroke, and hypertensive heart disease had similar high implant survival rates (98-100%) to patients without CVD (Heitz-Mayfield et al. 2018). According to the 2017 World Workshop Consensus (Schwarz et al. 2018), the major risk indicators of developing peri-implantitis were a history of chronic periodontitis, poor plaque control, and lack of regular maintenance after implant therapy. The evidence implicating systemic conditions as risk factors for peri-implant disease is inconclusive. Only limited evidence was present with respect to CVD or other systemic conditions(Schimmel et al. 2018; Schwarz et al. 2018). The current available epidemiological evidence with regards to the association between peri-implant disease/implant survival and CVD-related conditions was summarized in the Table 1.

In the present study, it was hypothesized that chronic inflammation at sites of peri-implant disease may induce systemic low-grade inflammation and increase the risk of cardiovascular disease via a potential infectious axis between the two diseases similar to that between chronic periodontitis and CVD. The purpose of this case-controlled design, cross-sectional study was to assess the relationship between peri-implantitis and CVD. The critical question is "does peri-implantitis impart an increased risk for future cardiovascular disease? ". Secondly, we compared the diagnostic value of systemic and peri-implant protein biomarkers and peri-implant microbial profiles for peri-implantitis in CVD and non-CVD patients. The null hypothesis was that patients with peri-implantitis are not at elevated risk for developing cardiovascular diseases (CVD).

Chapter III. Material and Methods

A. Study Design

In order to investigate the relationship between peri-implantitis and CVD, a case-controlled design, cross-sectional study was implemented based on a power calculation to satisfy the two-tailed confidence level of 95% and a power of 80% with a ratio of 2 to1 (Case to Control). Individuals without CVD were grouped as "Control', and participants in the "Case" CVD group were recruited only when the dental implants were placed prior to

CVD diagnosis. It was assumed the percentage of "Controls" exposed to the peri-implantitis is 40%, according to Derks et al., which was diagnosed based on the similar case definition of this study (detectable bone loss). With a hypothetical prevalence 67% of "Cases", the sample size was calculated using an open-source OpenEpi statistic software version 3.01 with a total sample size of 137 composed of 91 subjects in the "Cases", and 46 subjects in the "Control" group. This was in accordance with the *a priori* power calculation based on the comparison of pro-inflammatory biomarker interleukin-1β concentration among the two groups. It was assumed that IL-1β level in the "Cases" (CVD) group is 200±135 pg/ml, and the "Controls" (non-CVD) group is 130±135 pg/ml (Offenbacher et al. 2009; Gürlek et al. 2017; Renvert et al. 2017), and a total sample size of 134 (89 in the "Cases" group, and 45 in the "Controls" group with the allocation ratio of 2:1) was acquired to satisfy the 80% power at the 5% significance level. In order to test the hypothesis of this investigation, the time of peri-implantitis onset was evaluated by radiographic evidence. As a result, the subjects with peri-implantitis being observed after the diagnosis of CVD being diagnosed were excluded in this case-control study.

B. Study population

This study, conducted from April 2018 to June 2020, was approved by the Institutional Review Boards of the University of Michigan Medical School (Study ID: HUM00130676) and at the Department of Periodontics and Oral Medicine, School of Dentistry, University of Michigan in collaboration with the Frankel Cardiovascular Center, Michigan Medicine. Participants enrolled in the "Cases" CVD group were those with an established diagnosis of cardiovascular diseases (CVD) or atherosclerotic cardiovascular disease (ASCVD) including coronary heart disease, cerebrovascular disease, and peripheral artery disease after at least one dental implant placement; and those who had received interventions to prevent a subsequent CVD event or with a history of a cardiovascular event (myocardial infarction, stroke, stable or unstable angina, transient ischemic attack, or coronary or other arterial revascularization including coronary artery bypass graft or coronary transluminal angioplasty with or without a stent) after at least one dental implant placement and functioning for at least 6 months. The diagnosis of CVD was assigned by an internist or a cardiologist by the findings of symptoms, ischemic changes in ECG, findings in coronary angiography, and a history of a previous CVD event. All patients with the diagnosis of CVD were recruited by the screening process from the database of electronic health records from the Frankel Cardiovascular Center, Michigan Medicine and the School of Dentistry, University of Michigan. The "Controls" non-CVD group were patients with at least one non-mobile dental implant and in function for at least 6 months but without a diagnosis of CVD recruited by the same screening

process via electronic health records in the School of Dentistry, University of Michigan. Eligibility of inclusion criteria was confirmed through telephone interview and written informed consent was obtained prior to subject enrollment.

Inclusion Criteria

1. Adult subject age ≥ 25 years old
2. All subjects must have at least one implant non-mobile, in function for at least 6 months
3. For the "Cases" (CVD) group (i) who had established diagnosis of cardiovascular disease (CVD) or atherosclerotic cardiovascular disease (ASCVD) including coronary heart disease, cerebrovascular disease, and peripheral artery disease after at least one dental implant placement (ii) who had received interventions to prevent a subsequent CVD event or with a history of a cardiovascular event (myocardial infarction, stroke, stable or unstable angina, transient ischemic attack, or coronary or other arterial revascularization) ***after*** at least one dental implant placement
4. No known episode of peri-implant infection (mucositis/peri-implantitis) within the past year
5. No known mechanical complication affecting the implant restorations within the past year

Exclusion Criteria

1. Unable to comply with the study protocol
2. Head/neck radiotherapy, chemotherapy, or immunosuppressed therapy in the past 6 months
3. Pregnant or lactating female
4. > 2 weeks use of antibiotics in the past three months
5. Patients taking medications known to modify bone metabolism (e.g., IV bisphosphonates or oral bisphosphonates for more than 4 years, long-term use of corticosteroids, long-term use of hormone replacement therapy)
6. Drug addiction or intoxication other than habitual tobacco consumption
7. Previous treatment for periodontitis or peri-implantitis in the past 3 months

C. Clinical and radiographic assessment

The updated medical history was interviewed by constructed questionnaire and documented in the case report form. Medical history was collected including age, gender, ethnicity, height, weight, BMI, health history including diabetes mellitus (DM) and glycemic control, hypertension (systolic pressure >130 or diastolic pressure > 80 mmHg measured on various occasions or taking antihypertensive medications), dyslipidemia or metabolic disease, osteoporosis, rheumatoid arthritis or other major diseases), and life-style (smoking including data of dose/year and alcohol consumption including data of frequency/units). The medication list and the family medical history with regard to heart attack, stroke, cancer, diabetes, hypertension, etc. were also recorded. Physical assessments were performed including vital signs check (pulse and blood pressure), fasting plasma glucose test, extra-oral exam (communication, general physical status, face and muscle tone, thyroid, lymph nodes palpation) and intra-oral exam (color, contour, and texture of soft tissue, hard palate, tonsils, tongue, and mouth floor and general dental and occlusion exam).

Dental history was documented including the history of periodontal maintenance and the time of implant insertion, implant location, and potential history of treating peri-implantitis. All the implants were assessed by radiographic examination first to identify the "most bone loss implant" and verify the eligibility if the time of the placement of selected implant was prior to the time when the diagnosis of CVD was established by searching the electronic dental record in the University of Michigan or acquiring from the patient's dental record if possible.

Standardized long cone paralleling periapical digital radiographs of all the implants were taken if radiographs in the electronic dental record were taken longer than one-year previously or had significant changes in the pocket depth and tissue status since the last dental examination within one-year. Similarly, in the remaining natural teeth, multiple peri-apical radiographs were obtained if there were no recent radiographs within two years, or were updated in the periodontal susceptible patients if radiographs were taken more than one-year previously. The radiographic examination was performed using a paralleling long-cone technique with Rinn-type film holder (XCP-DS® Sensor Holder, Dentsply Sirona) and examined under an in-house computer software (MiPACS Dental Enterprise Viewer) to discern the bone loss apical to the most coronal portion of the intraosseous part of the implant (or anticipated level after initial bone remodeling) measured in millimeters to the first visible bone-to-implant contact (BIC) The implant surface topography type (e.g., bone or tissue level

implants) were determined based on the macro-design shown on the radiographs or acquired from the dental records in the dental school. If the implant system could not be identified, they were excluded from the stratified statistics on the implant surface category. All assessments were made by one self-calibrated examiner under the same viewing conditions.

Prior to the clinical assessment, collection of plaque samples and peri-implant crevicular fluid (PICF) around the targeted "diseased" or "healthy" implant and the gingival crevicular fluid (GCF) samples around the most-diseased or healthy natural tooth were completed to avoid the contamination of the PICF/GCF samples after the assessment of bleed upon periodontal probing. The process of sample collection was detailed in the section *D. Serum, subgingival plaque and PICF/GCF sample collection*

Clinical assessments were recorded at 6 sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) around the "most radiographic bone loss" implants. If the patient had received more than one implant, clinical data and PICF/plaque samples were collected only from the most bone loss peri-implantitis-affected implant. The clinical data collected included pocket depth (PD), clinical attachment level (CAL) with the reference of implant crown margin, modified plaque index (0, 1, 2, 3) (modPI) (Mombelli et al. 1987), modified gingival index (0, 1, 2, 3)(modGI) (Mombelli et al. 1987), BOP (+/-), and suppuration (+/-). Keratinized mucosa widths (KMW) were measured at buccal/lingual surface. Finally, the implant restoration type (fixed, removable, or hybrid), connection type (cement or screw-retained), stand-alone prosthesis or splinted, mobility (+/−), and the opposing arch (natural tooth or implant-supported prosthesis) were documented. All the clinical measurements were completed using a UNC-15 periodontal probe (Hu-Friedy, Chicago, IL, USA). Clinical photographs were acquired when possible. Clinical assessment further included a full-mouth periodontal chart to record PD, CAL, BOP (+/-), keratinized mucosa width (KMW), furcation involvement (grade 1, 2, 3) (Hamp et al. 1975), mobility, and occlusal evaluation (including any signs of parafunctional activities). Peri-implant status and overall periodontal status obtained from the clinical data were determined by the case definitions in accordance with the 2017 World Workshop (see *E. Case definition of peri-implantitis and periodontitis*) and stratified into subgroups for further statistical analysis.

D. Serum, subgingival plaque and PICF/GCF sample collection

Serum

Arterial blood samples were collected in BD Vacutainer® Serum 10 mL tubes (Becton Dickson and Company; Franklin lakes, NJ, USA) following the standard procedures suggested by the manufacturers from fasting patients (at least 8h). Whole blood samples were allowed undisturbed at room temperature for 30 mins post-collection, followed by tube centrifugation at 2700 rpm for 15 mins. Serum samples (approximately 1 milliliter) were immediately (within 30 mins) aliquoted into a labeled Eppendorf® polypropylene cryovial using pipette technique (multiple cryovials were obtained based on the volume of the blood draw) and placed into a cold box for transporting to the lab and stored at -80°C freezer until the analysis.

Peri-implant crevicular fluid (PICF)

The PICF samples were collected from the "most radiographic bone loss" implant per patient, either from the mesio-buccal aspect of the healthy implant site or the deepest implant pocket of the "most radiographic bone loss implant". Each implant crown was dried with sterile gauze, isolated using cotton rolls and dried with gentle air spray. The supra-gingival plaque was removed using a sterile currete avoiding damage to the soft tissues. PICF samples were collected using methylcellulose strips PerioPaper® (Oraflow Inc., Smithtown, NY, USA) gently placed into the cleansed and dried pocket for 1 min. PerioPaer® strips contaminated with blood were discarded and the site was re-sampled after 90s.The samples were transferred into labeled Eppendorf® polypropylene cryovials, and stored in a -80°C freezer for further analysis.

Plaque biofilm

Subgingival plaque biofilms were collected at the same implant site after the PICF collection to establish the relationship between biomarker and microbial profiles. Sterile paper points (size 25 taper Absorbent Points, Dentsply Sinora) were inserted apically into the peri-implant sulcus until meeting resistance. They were kept at the base of the sulcus/pockets for 30 seconds. The samples were immediately placed into labeled vials containing 500 µl of stabilizing buffer (RNAprotect™, Ambion, Austin, TX, USA) to prevent mRNA degradation during transportation. They were hand-shaken for 10s, then vortexed for 30s, and stored in a -80°C freezer until analysis.

Gingival crevicular fluid (GCF)

GCF samples were taken from 2 chosen sites from each patient. Based on the previous periodontal record and radiographic examination, one was collected from the most severe periodontitis site (deepest PD) in the subjects with chronic periodontitis and the other one was collected from a healthy site (mesio-buccal site of healthy tooth). Two healthy or gingivitis sites were randomly chosen in the subjects with healthy periodontal status. Prior to collection, supragingival plaque was removed using a sterile instrument. The sites were isolated using cotton rolls and dried using a short blast of air (not into the sulcus/pocket). GCF samples were collected using methylcellulose strips PerioPaper® (Oraflow Inc., Smithtown, NY, USA) gently placed and kept in the dried pockets for 30 seconds. PerioPaper® strips contaminated with blood were discarded and re-sampled after 90 s. Subsequently, GCF strips were stored in cryovials at -80°C until extraction for analysis.

E. Case definition of peri-implantitis and periodontitis

(1) The case definition of peri-implant health and peri-implant disease including peri-implant mucositis and peri-implantitis implemented in the current investigation followed the diagnostic criteria proposed in the consensus report of 2017 World Workshop (Berglundh et al. 2018; Renvert et al. 2018).

- Peri-implant health: (1) absence of peri-implant signs of soft tissue inflammation (redness, swelling, profuse bleeding on probing (BOP) (2) no increase in probing depth compared to previous examination (3) absence of further bone loss beyond crestal bone level changes resulting from initial bone remodeling
- Peri-implant mucositis (MU): (1) presence of peri-implant signs of inflammation (redness, swelling, line or drop of bleeding within 30 seconds following probing (2) absence of further bone loss beyond crestal bone level changes resulting from initial bone remodeling
- Peri-implantitis (PI): (1) presence of peri-implant inflammation (2) increasing probing depth (PD) compared to previous examinations (3) radiographic evidence of bone loss beyond crestal bone level changes resulting from initial bone remodeling. In the absence of previous radiographs, radiographic bone level ≥ 3 mm (apical of the most coronal protion of the intraosseous part of the implant) in combination with BOP and PD ≥ 6 mm.

  In order to discern the inflammatory burden of peri-implantitis, another cut-off threshold of peri-implantitis (BOP/suppuration and radiographic bone loss ≥2mm) was implemented in the analysis, (moderate to severe peri-implantitis) (Sanz et al. 2012; Derks et al. 2016). Further severity of peri-implant bone loss was analyzed statistically, including mild peri-implantitis (bone loss< 2 mm), moderate peri-implantitis (bone loss 2-4 mm) and severe peri-implantitis (bone loss> 4mm) lesions.

- Peri-implant disease (PID) was defined as chronic inflammation around dental implant, including peri-implant mucositis and peri-implantitis.

(2) The case definition and severity classification of periodontal health and periodontitis implemented in current investigation followed the diagnostic criteria proposed in the consensus report of 2017 World Workshop(Chapple et al. 2018; Lang and Bartold 2018; Papapanou et al. 2018; Tonetti et al. 2018).

Case definition

- Periodontal health: absence of clinical detectable inflammation, which including intact periodontium and a reduced periodontium presented with stability or disease remission after treatment
- Periodontitis: (1) ≥ 2 non-adjacent teeth presented with interdental CAL (2) Buccal CAL ≥ 3 mm with PD ≥ 3 mm detectable at ≥ 2 teeth (excluded conditions such as gingiva recession, caries, vertical fracture, etc.)

The purpose of stratified analysis of the classification (severity) of periodontal health and disease in the current investigation was to evaluate the impact of periodontitis disease severity of the remaining teeth onto the systemic inflammation burden accompanying with the peri-implant disease. If only one area (adjacent teeth) presented with interdental CAL, or teeth with periodontal stability/remission, the case was categorized as "periodontal healthy".

Classification of periodontitis

Staging: based on severity defined by the level of interdental CAL, radiographic bone loss (RBL) and tooth loss due to periodontitis, in combination with complexity to classify 4 stages of chronic periodontitis.

- Stage I: initial periodontitis
- Stage II: moderate periodontitis
- Stage III: severe periodontitis
- Stage IV: advanced periodontitis

	Greatest CAL	RBL	Toth loss	Complexity
Stage 1	1-2 mm	Coronal third <15%	No	Maximum PD ≤ 4 mm Mostly horizontal bone loss
Stage II	3-4 mm	Coronal third <15%	No	Maximum PD ≤ 5 mm Mostly horizontal bone loss

Stage III	≥ 5 mm	Extending to mid-third and beyond	≤ 4 teeth	PD ≥ 6 mm Vertical bone loss ≥ 3 mm Furcation involvement Class II or III
Stage IV	≥ 5 mm	Extending to mid-third and beyond	≥ 5 teeth	< 20 remaining teeth (10 opposing pairs) Need for complex rehabilitation due to masticatory dysfunction

Grading: based on the indirect evidence of disease progression including % bone loss/age and case phenotype (consistency of destruction and biofilm deposits) and two risk factors (smoking and diabetes). Additionally, the risk of systemic impact of periodontitis also was taken into consideration by the measurement of high-sensitivity C-reactive protein (hsCRP). (the direct evidence was not available in all samples)

- Grade A: slow rate of progression
- Grade B: moderate rate of progression
- Grade C: rapid rate of progression

	% bone loss/age	Case phenotype	Smoking	Diabetes	hsCRP
Grade A	< 0.25	Heavy biofilm with low destruction	Non-smoker	Normoglycemic/no diabetes	< 1 mg/L
Grade B	0.25-1	Destruction commensurate with biofilm	< 10 cigarettes/day	HbA1c < 7.0% in diabetic patients	1-3 mg/L
Grade C	> 1.0	Destruction exceeds expectation given biofilm	≥ 10 cigarettes/day	HbA1c ≥ 7.0% in diabetic patients	> 3 mg/L

F. Laboratory analysis

Serum Analysis

High-sensitivity C-reactive protein (hsCRP) was measured in the serum samples using RayBio® Human CRP ELISA assay (RayBiotech, Inc., Norcross, GA, USA). Optical density was measured at 450 nm, and the concentration was determined by interpolation from a calibration curve of known concentrations with a dilution factor of 1000X. The second part of serum samples were analyzed using human custom Quantibody® (multiplexed sandwich ELISA-based) Arrays (RayBiotech, Inc., Norcross, GA, USA) to detect and quantify the cytokine levels including interleukin-1 beta (IL-1β), interleukin-6 (IL-6), plasma tumor necrosis factor-alpha (TNF-α), matrix metallo-proteinase-8 (MMP-8), and osteoprotegerin (OPG) according to the manufacturer's

protocol. After the capture of antibodies and incubation, the target cytokine is trapped and arrayed on the glass plates. By addition of the streptavidin-conjugated Cy3 equivalent dye, the laser scanning and multiplex detection was completed. Separately, fibrinogen was measured using Human Fibrinogen SimpleStep ELISA® kit (Abcam, Cambridge, UK) via high-sensitivity RabMab® antibodies to detect the lower amounts of fibrinogen in the serum samples. The assay was performed according to the manufacturer's instructions. Optical density was measured at 450 nm using an EZ Read 400 Microplate Reader (Biochrom, Holliston, MA, USA). Finally, the remaining serum samples were analyzed after a 1:5dilution with isotonic saline with the chemistry automated analyzer and regent kits (ADVIA® 1800 Chemistry System, Siemens Healthcare Diagnostics Inc., IL, USA) for the lipid panel including total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides. The total cholesterol/HDL, LDL/HDL ratio was automatically calculated.

PICF/GCF Analysis

A 20 µl extraction solution containing 10g/ml aprotinin, 1mM phenylmethylsulfonyl fluoride, and 0.1% human serum albumin in phosphate-buffered saline (PBS, pH 7.4) was pipetted directly onto the cellulose portion of each PerioPaper® strip and secured at the top of a 12*75 mm polystyrene culture tube using a cap to hold it in place. After centrifugation at 2000 rpm at 4°C for 5 min, each strip was washed five times to yield a total elution volume of 100 µl. The samples were stored at -80°C until the antibody array quantification. The samples were thawed and quantitative assessments of biomarker expression in PICF and GCF samples were performed using custom human Quantibody® Arrays (RayBiotech, Inc., Norcross, GA, USA). Targeted biomarkers included 11 kinds: hsCRP; pro-inflammatory and angiogenic biomarkers including interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), plasma tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF); T-cell modulator: interleukin-17 (IL-17); chemokine: interleukin-8 (IL-8); inflammation mediator and proteolytic enzymes: matrix metallo-proteinase-8 (MMP-8), and biomarker for bone metabolism: osteoprotegerin (OPG) and tissue inhibitor of metalloproteinase-2 (TIMP-2), and myeloperoxidase (MPO). Prostaglandin E2 (PGE2) was separately analyzed by the ELISA assay as described above. The validity of the standard curve for each protein was confirmed by visual inspection and managed the outliners lower than the limit of detection (LOD) as the lowest value as LOD.

Microbial analysis

Plaque samples absorbed onto paper points were sent out for a commercial laboratory (Zymo Research, Irvine, CA) to process and analyze by 16S rRNA next-generation sequencing (NGS) technique (ZymoBIOMICS® Targeted Metagenomic Sequencing Service). The mock microbial composition of the ZymoBIOMICS® microbial community positive standard measured in this project was demonstrated in Supplemental Figure 1. Blank extraction samples were used as negative controls. The total DNA extraction was completed by ZymoBIOMICS®-96 MagBead DNA Kit. The 2 µl per sample out of 50 µl DNA extract were prepared for targeted sequencing with the Quick-16S™ NGS Library Prep Kit in real-time PCR machines to control cycles and limit PCR chimera formation. With the Quick-16S™ primer sets V3-V4, the final library was sequenced on Illumina® MiSeq™ with a v3 reagent kit (600 cycles). Unique amplicon sequences were inferred from raw reads using the Dada2 pipeline (Callahan et al. 2016). Next, the taxonomy was assigned with the Zymo Research Database as a reference. Composition visualization, alpha-diversity (OTU diversity within sample using observed species), and beta-diversity (OTU diversity among sample communities) analyses were performed. The abundance among different groups were identified by linear discriminant analysis (LDA) effect size algorithm (LEfSe) (Segata et al. 2011). The "Absolute Abundance Quantification" was made possible by calculating the number of gene copies per microliter in each DNA samples using an equation generated by the plasmid DNA standard curve. The composition (%) of the total sample was calculated to evaluate the patterns of clusters of microbial distribution among samples, and represented in the taxonomy heatmaps. The genomic DNA was stored at 4℃ until next step of quantitative real-time PCR to analyze the targeted species shown to be abundant in the 16S NGS analysis. In addition, the interested species *Staphylococcus aureus* (Sau31 gene) was also targeted. Real-time quantitative polymerase chain reaction (RT-qPCR) were performed by a commercial laboratory (Microgen Diagnostics, Texas, USA) on the LightCycler 480 II running Lightcycler 480 software (Roche Molecular System, Inc.). Proprietary lab-developed probe-based assays were performed using a Ct cutoff of 35 cycles limiting off-target amplification detection and primer dimers with custom primers. The bacterial load was determined using standard curves of qPCR targets with known copy numbers and absolute copies were calculated from sample volume.

G. Data Analysis

Patient-reported or physician-reported diagnosis of cardiovascular disease (CVD) was reported by 8 categories: (1) Coronary heart disease (CHD) including myocardial infarction (MI), angina pectoris, congestive heart failure (CHF) (2) Cerebrovascular disease including stroke and transient ischemic attack (3) Peripheral artery

disease (PAD) (4) rhythm disorders (pacemaker implant) (5) valvular disease (6) subclinical atherosclerosis (7) aortic disease including thoracic or abdominal aortic aneurysm and (8) Cardiomyopathy and heart failure, venous disease, etc. For subgroup analysis, age was divided into 5 age categories of every 10-years of interval. To assess potential confounders, selected confounding factors in the structured questionnaires included: demographic factors (age, gender), cardiovascular risk factors (hypertension, diabetes mellitus (DM), high cholesterol, overweight and obesity (BMI), familial cardiovascular risk factors (hypertension history, DM history, and CVD history), behaviors (smoking and alcohol drinking).

Among them, DM, hypertension, and high cholesterol were also confirmed by the use of medications for each specific disease. Smoking was categorized into three subgroups including current-, ex-, and never-smoker. The years of quitting smoking in ex-smokers were divided into 4 types (within 5 years, 5-10 years, 10-15 years, and >15 years) (Table 2). Alcohol consumption was further categorized into dose-dependent 4 subgroups based on the risk threshold for the CVD (100g per week, which was equivalent to 7.1 units in US) (Wood et al. 2018) including social drinker, < 7 units/week, 7-14 units/week, and >14 units/week. Body mass index (BMI) was computed by dividing the weight in kilograms by the square of height in meters, and categorized into underweight (BMI< 18.5), normal weight (BMI: 18.5-24.9), overweight (BMI: 25-29.9), obesity (BMI: 30-39.9); and the obesity was further subdivided into class I (BMI 30 to <35), class II (BMI 35 to <40), and class III: severe obesity (BMI> 40)(World Health Organization 1997). Fasting glucose level was categorized into 4 types according to the corresponding HbA1C level to reflect glycemic control, including < 100 mg/dl (normal), 100-125 mg/dl (prediabetes), 126-153 mg/dl (well-controlled DM), and 154-183 mg/dl (moderate-controlled DM), and > 183 mg/dl (poor-controlled DM). Metabolic syndrome was determined when any 3 of the following 5 risk factors were met (Grundy et al. 2004): (1) fasting glucose ≥ 100 mg/dL or undergoing drug treatment for elevated glucose (2) HDL < 40 mg/dL in males or < 50 mg/dL in females or undergoing drug treatment for reduced HDL-C (3) Triglycerides ≥ 150 mg/dL or undergoing drug treatment for elevated triglycerides (4) BMI higher than BMI ≥ 25 (Overweight) which is equivalent to waist circumference (WC)> 102 cm in males or WC >88cm in females (5) systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or undergoing drug treatment for hypertension in a patient with a history of hypertension. Dyslipidemia from the lipid panel analysis was diagnosed based on the following standards(Grundy et al. 2019): (1) total cholesterol (< 200 mg/dL= normal, 200-239 mg/dL= borderline high, ≥ 240 mg/dL= high) (2) low-density lipoprotein cholesterol (LDL-C) [< 100 mg/dL= optimal, 100-129 mg/dL= above optimal, 130-159 mg/dL= borderline high, 160-189 mg/dL= high (hypercholesterolemia), ≥ 190 mg/dL= very high (severe hypercholesterolemia)] (3)

high-density lipoprotein cholesterol (HDL-C) (< 40 mg/dL= low (suboptimal) in men (< 50 mg/dL in women), 40-60 mg/dL= borderline in men (50-60 mg/dL in women), and >60 mg/dL= high (optimal) (4) triglycerides [< 150 mg/dL= normal, 150-199 mg/dL= borderline high, 200-499 mg/dL= high (hypertriglyceridemia was defined as 150-499 mg/dL), >500 mg/dL= very high (severe hypertriglyceridemia)]. Furthermore, the ratio of total cholesterol/HDL was considered normal as < 4.5 in men or < 4.0 in women, and risk threshold for heart disease and stroke was > 5.0 in men and > 4.5 in women; and the ratio of LDL/HDL was normal as < 3.0 in men or < 2.5 in women, and risk threshold was > 3.5 in men and > 3.0 in women. Lastly, very-low-density lipoprotein cholesterol (VLDL-C) was estimated by one fifth of the triglycerides level, and an elevated VLDL-C level (≥ 30 mg/dL) is assessed as another important risk factor for CHD. A further step of analysis was executed to explore the 10-year risk index for ASCVD using the algorithm developed by American College of Cardiology(Lloyd-Jones et al. 2019). The determinants included age, gender, race, SBP, total cholesterol, HDL-C, history of DM, smoking habits, and if patient was on hypertension treatment; and the 10-year ASCVD risk can be categorized as low-risk(<5%), borderline risk(5% to 7.4%), intermediate (7.5% to 19.9%), and high (≥ 20%). Lastly, cardiometabolic disease was classified into 4 stages(Guo et al. 2014)

Stage 1: Have one or two of the following risk factors:
- a. high waist circumference (≥112 cm in men, ≥88 cm in women)
- b. elevated blood pressure (systolic≥130 mmHg and/or diastolic =85 mmHg) or on anti-hypertensive medication
- c. reduced serum HDL cholesterol (<1.0 mmol/L or 40 mg/dL in men; <1.3 mmol/L or 50 mg/dL in women) or on medication
- d. elevated fasting serum triglycerides (≥1.7 mmol/L or 150 mg/dL) or on medication

Stage 2 (Metabolic Syndrome or Prediabetes): Have only one of the following three conditions in isolation
- a. Metabolic Syndrome based on three or more of four risk factors: high waist circumference, elevated blood pressure, reduced HDL-c, and elevated triglycerides
- b. Impaired Fasting Glucose (IFG; fasting glucose ≥5.6 mmol/L or100 mg/dL)
- c. Impaired Glucose Tolerance (IGT; 2-hour glucose ≥7.8 mmol/L or140 mg/dL)

Stage 3 (Metabolic Syndrome+ Prediabetes): Have any two of the following three conditions:
- a. Metabolic Syndrome
- b. IFG
- c. IGT

Stage 4 (T2DM and/or CVD): Have Type 2 DM (T2DM) and/or CVD:
- a. T2DM (fasting glucose ≥126 mg/dL or 2-hour glucose ≥200 mg/dL or on anti-diabetic therapy)
- b. active CVD (angina pectoris, or status post a CVD event such as acute coronary artery syndrome, stent placement, coronary artery bypass, thrombotic stroke, non-traumatic amputation due to peripheral vascular disease)

The periodontal status was evaluated in two ways. First, the presence of periodontitis was dichotomized into negative or positive periodontitis by the case-definition of 2017 World Workshop which was the presence of ≥ 2 teeth with interdental CAL. Second, subjects were categorized into 4 periodontitis groups based on the classification of "Staging": stage 1 to stage 4. Lastly, periodontitis-associated covariates were categorized by the "Grading" and "Extent". Oral health factors including the frequency of maintenance was dichotomized into less

or more than once every 6 months, and the number of missing teeth was categorized into three ordinal scales including low (0-4), medium (5-9). and high (≥10 teeth).

H. Statistical analysis

Established diagnosis of CVD ("Cases") was the outcome variable in dichotomous format. The presence of peri-implant disease was the main exposure variable. All the "Cases" and "Controls" were characterized as frequency (n) and percentage (%). A binominal logistic regression was performed to compare the two groups. A descriptive analysis of quantitative variables was reported in the measures of central tendency (mean, median) and dispersion (standard deviation, interquartile range); the bivariate analysis with a normal distribution was performed using Student's *t* test or ANOVA. Continuous parameters were tested using Mann-Witney test or Krunskal-Wallis H test to compare groups if the p-value was <0.05 in the Shapiiro-Wilk test.

Median values were chosen as cut-off points to categorize dichotomized variates; then the crosstab analysis was implemented to analyze sensitivity and specificity for the disease prediction. Multivariate logistic regression was used to evaluate the adjusted odds ratio (ORs) of the association between peri-implantitis and CVD.

For fitting into the final model, first demographic variables were entered, Next, the systemic health and oral health-related variables were entered hierarchically. By comparing the explanatory power of model (fitness of evaluated by a change of -2 log likelihood) and the significance level < 0.05, variables contributing to the change of association were retained in the final logistic regression model. Additional subgroup analyses were performed to evaluate the impact of individual risk factors modifying the association. Subgroups included age, gender, smoking, alcohol, hypertension, DM, BMI, lipid profile were analyzed. Significance level was defined as P<0.05 and the statistical analysis was completed by the SPSS 24.0 statistical software package for MAC (IBM, Amorak, NY, USA).

Chapter IV. Results

I. Case-control study

A. Clinical parameters

A total of 132 patients met the inclusion criteria of implant placement prior to the time of CVD diagnosis. After examining the radiographic evidence of progressive bone loss to discern the peri-implantitis status, 4 subjects in the CVD group were excluded from statistical analysis since the time of peri-implantitis occurrence was not clear relative to the time of CVD diagnosis (diagnosis of CVD and the time of implant placement were within 2 years). In other words, no radiographic before the time of the diagnosis of CVD was available to detect the bone loss after restoration. As a result, a "Case" group (CVD group) comprised of 82 participants and a "Control" group (non-CVD group) with 46 participants were included in the final statistical analysis.

Demographic variables were demonstrated in Table 2, including the chi-square test to describe the strength of relationship and the univariate logistic regression for further step-wise prediction modeling. The majority of the diagnosis of cardiovascular disease in the CVD group was coronary heart disease (29.3%), followed by arrhythmias (28%), cerebrovascular disease (14.6%), and peripheral artery disease (14.6%). Among the patients with arrhythmia, half of the participants received pacemakers (14.6% in total CVD group) (Table 3).

Mean value of age among the CVD group was significantly higher than the control group (75.0 ± 8.3 vs 69.3 ± 10.1, $p<0.01$). The significance was also observed among different categories of age (Table 2). Gender was not shown to be significantly different between the two groups, however the majority of CVD group was composed of males with a ratio of 2 to 1. Smoking history was significantly different between the two groups. Although the percentage of current smokers was similar between two groups, the percentage of ex-smoker was significantly higher in CVD group (57.3%) compared to the non-CVD group (34.8%) ($p= 0.03$). Among the ex-smokers, the composition of different years of smoking cessation was similar between two groups, and the majority of individuals in this group quit smoking more than 15 years previously ($p= 0.52$). Alcohol consumption was similar between the two groups. Family history of heart attack was reported in 58.5% patients in the CVD group which was significantly higher than the 30.4% in the control group ($P=0.01$). Family history of stroke, hypertension, diabetes, and cancer didn't show significant differences favoring the CVD group. With regard to the cardiovascular risk factors, CVD cases exhibited a significantly higher prevalence of hypertension than controls (59.8% vs 39.1%, $p=0.03$), whereas there was no significant difference in other variates including BMI, metabolic syndrome, diabetes, self-reported cholesterol, osteoporosis, and rheumatoid arthritis(RA) (Table 2). However, the prevalence of metabolic syndrome and diabetes was higher in the CVD group, which was in

accordance to the general trend of fasting glucose level and the lipid profile shown in the Table 4. Self-reported high cholesterol was found in the CVD group (46.3% vs 34.8%). There was a higher percentage of participants in the CVD group exhibiting low (suboptimal) HDL-C level (34.3%) compared to the control group (13.5%) ($p=0.07$). Interestingly, there was higher percentage of participants in the healthy group (51%) with above normal LDL-C levels compared to 22% in CVD group($p<0.01$). This may be explained by the prevalent consumption of anti-dyslipidemia (statin) medications in the CVD group (60% in CVD versus 28% in non-CVD group). Most importantly, the 10-year ASCVD risk assessment was shown to be significantly higher in the CVD group at 68.7% compared to the control group (32.4%). Participants in CVD group were uniformly stage 4 of cardiometabolic disease. However, it was noteworthy that one third of the non-CVD group presented with stage 3 cardiometabolic disease, which was related to the lipid profile. Lastly, participants in CVD group exhibited a much higher percentage of multiple co-morbidities compared to the healthy group. One-third had at least one co-morbid factors, one-fifth had two co-morbid factors and one-third had 3 or more co-morbid factors. The most common co-morbid factor was metabolic disease, followed by obesity, diabetes, depression, osteoarthritis, and cancer.

Prevalence of Peri-implantitis

The prevalence of peri-implant health defined by the absence of signs of soft tissue inflammation (lack of BOP) and marginal bone loss beyond the initial physiologic bone remodeling was found in 9.8% (n=12) of the CVD group and 8.7% in the control group (n=4). The prevalence of peri-implant mucositis (no detectable bone loss beyond the initial physiologic bone remodeling) was found in 28.9% of the CVD group (n= 37) and 34.8% (n=16) in the control group. The prevalence of peri-implantitis defined by the presence of additional bone loss beyond the initial physiologic bone remodeling was found to be 65.9% (n=54) in the CVD case group and 56.5% (n=26) in the control group. The crude odds ratio from the bivariate logistic regression for the association between peri-implantitis and CVD was 1.48 (95% CI = 0.71 to 3.11, p= 0.30). Alternatively, a significant association was found between peri-implantitis and CVD (crude odds ratio= 2.18, 95% CI= 1.02 to 4.67, p= 0.04) when compared the prevalence of moderate to severe peri-implantitis (radiographic bone loss$\geq$ 2mm). The prevalence of moderate to severe peri-implantitis, 48.8% (n=54) in the CVD group, was significantly higher than the 30.4% (n=14) in the control group. The overall peri-implant disease (peri-implant mucositis and peri-implantitis) exhibited a similar prevalence between the two groups (90.2% vs 91.3%, p=0.84). The prevalence of peri-implantitis based on different cutoff threshold of bone loss is shown in Table 5. Higher peri-implant

pocket depth (PPD) and BOP% was found in the CVD group ($p > 0.05$), especially with respect to bone loss> 4 mm.

The CVD group exhibited a significantly higher incidence of periodontitis (76.4%) compared to the healthy control group (48.9%) with an OR= 3.4 (95% CI= 1.52 to 7.52, $P<0.01$) (Table 6). The majority of disease severity observed in both groups was generalized Stage 2. Grade C was demonstrated in both groups with a prevalence of 71.8% in CVD group and 68.2% in the control group when the hsCRP level was considered. On the basis of indirect evidence (radiographic bone loss) and other direct evidence (e.g., case phenotype of biofilm deposition or smoking habits), Grade B was the majority finding, and a trend towards higher grades were observed in the CVD group ($p=0.01$).

Tooth loss was significantly higher in the CVD group. One-third of the participants lost more than 10 teeth, and another one-third lost 5-10 teeth. In the control group, half of the participants lost less than 5 teeth ($p< 0.01$). Implant-supported overdentures were more prevalent in the CVD group (14.6% vs 2.2%). Full edentulism in the CVD group was also significantly higher compared to the control group (12.2 vs 2.2%, $p= 0.05$). Interestingly, the frequency of maintenance among two groups showed no difference with similar prevalence between the episodic and the regular subgroups ($p=0.23$).

Stratified analyses were performed based on different strata of age population. The association between moderate to severe peri-implantitis (radiographic bone loss ≥2mm) and CVD stratified by age were presented in Table 7. Without adjusting for any other factors (except gender), the estimates of odds of the association were 2.31 (95% CI= 1.06 to 5.02, $p=0.04$) in the total population (range: 43-91 year old); and the gender-standardized significant association mainly existed in the 60-80 years old (OR= 2.46, 95% CI= 0.94 to 6.47, $p=0.06$). All age strata did not show a significant association after adjusting for smoking, hypertension, and family history of heart attack. Another stratified analyses was performed on the impact that DM and metabolic syndrome brought to the odds ratio for cardiovascular disease. Crude odds ratio for developing CVD was found to be statistically significant restricted to the strata of patients without DM (OR=2.29, 95% CI= 0.96 to 5.47, $p=0.05$); interestingly, the crude odds for CVD was significantly higher among subjects with metabolic syndrome (OR=3.5, 95% CI= 1.01 to 12.12, $p=0.04$). However, after adjusting for age, the significance was not observed for either effect.

For the results of step-wise multivariate logistic regression, at the first layer of regression model, odds ratio of moderate to severe peri-implantitis (radiographic bone loss ≥2mm) for CVD adjusting for single confounder including smoking (including quitting-year), alcohol, diabetes mellitus, BMI, metabolic syndrome, HDL level, hypercholesterolemia, or comorbidities remained significant. Among relevant covariates, age, hypertension, family history of heart attack, smoking, cLDL level, ASCVD risk, absence of periodontitis, and the number of tooth loss significantly impacted the association. Periodontitis and ASCVD had a dose-responsive effect. By fitting all potential risk factors into the final model of the moderate to severe peri-implantitis, a significant association was not observed after multivariable adjustments (Table 8).

B. Biomarker Analyses

Serum Biomarkers

Seven serum biomarkers were tested to evaluate the association between peri-implant disease and CVD. The mean (median) values and significant differences between non-CVD and CVD group from the Kruskal-Wallis test results were presented in Table 9. The result showed that IL-1β, TNF-α and fibrinogen were significantly higher in the CVD group compared to the non-CVD group (CVD vs. non-CVD: 97 vs 58.4 pg/ml, 104 vs. 56.5 pg/ml, 86.2 vs. 62.3; respectively). Although not statistically significant, IL-6 and OPG demonstrated a trend of higher levels of detection in the CVD group. Conversely, the mean concentration of hsCRP was higher in the non-CVD group (CVD vs. non-CVD: 6.4 vs. 7.7 pg/ml).

In the stratified subgroup analyses, the serum hsCRP concentration in the CVD group was higher than the non-CVD among patients who had taken cholesterol-lowering medications (mainly statin therapy). Conversely, hsCRP was higher in the non-CVD group compared to CVD group among participants not taking statin therapy. The strata of participants with normal LDL-C level had similar hsCRP concentrations. However, hsCRP was higher in the non-CVD group restricted to participants with borderline to high cholesterol (LDL-C) (Table 10).

The diagnostic ability was further assessed using chi-square analysis to predict the outcome of disease (CVD) based of the cut-off point (median) of proteins. Receiver operating characteristic (ROC) curve analyses were

performed to further assess at the continuous-variate level. Both tests demonstrated a significantly fair accuracy of disease prediction with TNF-α (AUC 67%) and fibrinogen (AUC 65%) for CVD (Table 11) (Figure 1). The results of Chi-square analysis based on the dichotomized data showed that IL-1β, OPG, and TNF- α were promising predictors for the CVD (sensitivity: 58.6%, 86%, 60% and specificity: 63.2%, 45.2, 65.8%; respectively) (Table 12). Further subgroup analysis was implemented to evaluate the predictive value of serum biomarkers in different subsets of chronic inflammation around dental implants. The results were summarized in Table 13. Fibrinogen was higher in the moderate to severe peri-implantitis group compared to the healthy implants (p=0.08), and CVD group was generally higher than the non-CVD group. A similar trend was observed in the IL-6, TNF-α, and OPG when compared with peri-implant inflammation in the healthy sites. Serum biomarker levels were also evaluated to understand the association with the ASCVD risk classification among participants in the current investigation. IL-6 and TNF-α demonstrated a dose-response association (p> 0.05). The lower the protein level, the lower the 10-year ASCVD risk (Table 13).

PICF analysis

128 implants were evaluated for the PICF. The results of the 12 biomarkers were shown in Table 14. TNF-α exhibited a significantly higher detection level in the CVD group (p=0.05). The ROC curve analysis proved that PICF TNF- α was a predictor for CVD, but with poor accuracy of prediction (AUC=59%) (Figure 2). Other PICF biomarkers including MMP-8, MPO, and IL-17 seem to be higher in the CVD group but without a significant difference (p>0.05). On the contrary, the level of PICF CRP, PGE2, IL-6, IL-8, TIMP-2 and VEGF appeared to be higher in the non-CVD group.

In the subgroup analysis, MMP-8 proved to be significantly higher in the moderate to severe peri-implantitis (bone loss≥2mm) (3468.7 vs 3117.3 pg/ml, p=0.05). PGE2, IL-6, IL-17, MPO, OPG, TIMP-2, and TNF-α demonstrated the same trend. The abundance of these biomarkers displayed a higher level in the peri-implantitis subgroup without reaching significance. The local inflammation which manifested as peri-implant disease proved to be significantly associated with IL-1β, MMP-8, and TIMP-2 (p< 0.05). MMP-8 was elevated in the CVD group when compared to the non-CVD group (Table 15).

GCF analysis

Table 16 shows the mean values of GCF proteins collected from those teeth with periodontitis and teeth with healthy periodontium. in. CRP was higher in subjects with periodontitis compared to subjects with a healthy periodontium (293.5 vs 276.0 pg/ml). A similar trend was also found in IL-6, MMP-8, and OPG. Only IL-1β was statistically significantly higher collected from the periodontitis sites compared to the periodontal healthy sites (mean 160.2 vs. 119.3 pg/ml, $p<0.01$). VEGF exhibited a borderline significant difference (mean 97.5 vs. 73.0 pg/ml, $p=0.07$).

Comparison of GCF levels between case and control groups at different sites was shown in Table 17. In the subjects with periodontitis, either at sites of periodontitis or healthy periodontium, local gingival crevicular fluid TNF- α was higher in the CVD group. This trend with healthy periodontium, failed to reach significance. This pattern was not observed in other biomarkers. The predictability of GCF biomarkers associated with CVD was found in IL-6 collected from teeth with periodontitis in the dichotomized chi-square analysis, which is 5.7 in odds ratio (95% CI: 1.5-21.9, $p<0.01$). TNF-α collected from periodontitis teeth or healthy teeth in subjects with periodontitis was strongly associated with CVD cases with a sensitivity of 48.2% and specificity of 82.6% ($p=0.01$). The ROC curve analysis was illustrated in the Figure 3. If we compared peri-implantitis alone, only GCF OPG was found to be significantly higher in the moderate to severe peri-implantitis subgroup collected from periodontitis teeth (52.2 vs 26.6 pg/ml, $p< 0.04$) even though the dichotomized prediction may not reach significance ($p=0.13$) (Figure 4).

C. Microbial DNA profiles

A total of 16 million (1.62E+07) sequence reads were obtained from the 16S next-generation sequencing (NGS) analysis. After quality trimming and filtration, there were 14243 final unique sequence reads. Taxonomy composition at the Phylum and Class level among case and control group were shown in supplemental Figures 2 and 3 and Supplemental Table 1. Peri-implant pocket microbial community composition in the CVD group revealed higher percentages of *Bacilli, Actinobacteria,* and *Gammaproteobacteria*. Non-CVD group exhibited a higher percentage of *Fusobacteria* and *Clostridia*. The most common species in the total population was *Fusobacterium nucleatum* (13.6%), followed by *Streptococcus mitis-oralis-sanguinis* (4.6%), *Parvimonas mica* (3.4%), *Streptococcus intermedius* (3.4%), and *Veillonella parvula* (3.1%) (Table 18). The microbial composition individually in the case and control groups is shown in Table 19. *Haemophilus parainfluenzae* was

detected significantly more frequently in the CVD group (2.7 % vs 0.58%), and *P. gingivalis* was also found higher in the CVD group than the control (1.7% vs 1.0%). Contrastingly, *Alloprevotella tannerae* appeared with higher percentage in the healthy group, especially the healthy implant subgroups (1.8% vs 1.4%, case vs control, respectively). Linear discriminant analysis (LDA) algorithm (LEfSe) was used to estimate the effect the size of each differentially abundant trait, which ranks the phylotypes based on the relative differences between CVD and non-CVD groups, taking into account the variability and discriminatory power**.** The significantly abundant species discerned by the LEFse analysis were shown in the Figure 5. The CVD-related cluster was shown to be more associated with facultative anaerobic *Actinomyces*, and black-pigmented, Gram (-) anaerobic rods, *Prevottella pallens*. The most commonly found periodontal pathogens were summarized in Table 20.

The "absolute abundance" generated from the NGS analysis was expressed in gene copies per microliter of each sample. The average gene copies per ml was higher in the samples among the CVD group ($1.4362x10^6$/ml vs 1.0029x106/ml, p=0.27) (Figure 6). The alpha diversity comparing the diversity within samples is shown in Figure 7. The median of the number of observed species was similar between two groups. The CVD group presented with a deeper sequencing depth, which represented more taxa being identified at lower abundance. Beta diversity compared the diversity between samples, and is represented by a distance matrix algorithm (Principal Coordinates Analysis, PCoA). UniFrac plots were used to demonstrate the complex phylogenetically. This measured the phylogenetic distance between sets of taxa in the phylogenetic tree. samples with similar microbial composition profiles and closer to each other which represents the relatedness/similarity of samples, or clustering among samples. It was evident that CVD group exhibited a higher microbial diversity (Figure 8); and non-CVD group demonstrated a more clustered and centered profile.

Finally, subgroup comparison is demonstrated in Figure 9. The average absolute abundance (gene copies/ml) was significantly lower in the non-CVD group with healthy implants ($4.3x10^5$/ml) compared to the other three groups [significant difference compared to CVD + peri-implantitis group ($14.5x10^6$/ml) in Games-Howell post-hoc test, p=0.03]. No species stood out for the LEFse analysis among 4 groups, only two species *Lectotrichia goodfellowiii* and *Pseusomonas fluorescens fragi* were significantly more abundant in the non-CVD with healthy implant group (Figure 10). The heatmap of 4 groups is presented in supplemental Figure 4. *Actinomyces oris*, *Prevotella pallens*, and G(-) anaerobic cocci *Veillonella*, and *Fusobacterium subspecies periodonticum* were also prevalent in the peri-implant disease combined with CVD group in the comparison

between CVD and non-CVD when the analyses only compared among peri-implant disease (or peri-implantitis) (Figure 11, 12)

Beta-diversity among subgroups between case and control revealed a consistent observation that microbial profile in the subjects with CVD was more diverse; and the microbial profile of peri-implantitis also exhibited a higher dissimilarity compared to the healthy implant sites (Figure 13). This pattern of diversity of microbial profile comparing CVD to the non-CVD group was also observed in the peri-implant diseases (Figure 14).

As a result, the 3 most common periodontopathogenic species *P. gingivalis, T. forsythia, F. nucleatum* found in the 16S sequencing results and *S. aureus* were selected for real-time qPCR to detect absolute bacteria counts for subgroup analysis. The Kruskal-Wallis test of bacterial load comparing case and control and subgroups was performed after the normality test (Table 21, 22). The absolute counts of these 4 species were not significantly different between the "case" and "control" groups. However, *P. gingivalis* demonstrated a trend towards higher counts in the CVD group. Peri-implant pockets in the periodontitis subgroup harbored higher *P. gingivalis* compared to the healthy periodontal subgroup. The mean amount of gene copies of *F. nucleatum* was significantly higher in peri-implantitis group (radiographic bone loss ≥ 2mm) than the healthy group ($p<0.05$). A significant difference was also found between healthy implant and peri-implant diseases. *T. forsythia* was only found to be significantly higher in the severe peri-implantitis (radiographic bone loss >4 mm) group than the healthy implants (3.2E+07 vs 1.1E+07, $p=0.01$). The median and data dispersion (range) of these 4 species among healthy implant, mucositis, and peri-implantitis is demonstrated in Figure 15. *S. aureus* appeared to be an opportunistic species in the current investigation since only 4 samples found *S. aureus* at the DNA account of 1.64E+04 gene copies. The characteristics of those patients were demonstrated in Table 23. There was a trend of *S. aureus* associated with more severe bone loss and deeper peri-implant pockets and suppuration.

II. Implant-level microbial and biomarker analysis

An additional 10 patients with peri-implantitis were recruited to enhance the power of microbial profiles and biomarker discovery. A total of 142 participants were recruited and reported in this implant-level analysis. Subgroup analyses of microbial profiles established using 16S NGS technology were performed to compare

different peri-implant pocket depths (PPD), bone loss, presence/absence of suppuration, dentate/edentulous patients, and different stages of periodontitis. Absolute abundance (gene copies per ml) and PPD presented with a dose-responsive correlation, which was significantly higher in moderate pockets (4-6 mm), and even higher in deep pockets (≥ 7 mm) (Figure 16). The alpha diversity demonstrated higher species number can be found at peri-implant sites with moderate PPD than the deep PPD which was mainly composed by the anaerobic organismas (*Corynebacteriaceae, Staphylococcae, Carnobacteriaceae, Neisseriaceae, Leptotrichiaceae, Burkholderiaceae, Pasteurellaceae,* and *Gammaproteobacteria*). Deeper pockets harbored mainly *Prevotellaceae, Eubactteriaceae* and *Erysipelotrichia* and shallow pockets harboring more *Euryarchaeota*, Gram (+) *rods, cocci, Actinobacteria, and Rickettsiales* instead (Figure 17). LEfSe analysis revealed the abundance species in three groups was illustrated in the Supplemental Figure 5. It can be related to the difference disclosed in the LDA Score comparison (LEfSe analysis) of bone loss level among subjects with peri-implantitis (Figure 18). *Tannerella forsythia, Bacteroidales*, and *Bacteroidia* was found to be significantly abundant in the subjects with bone loss > 6 mm. In contrast, *Haemolysans sanguinis taiwanensis, Gemella, Bacillales* family, and *Lachnoanaerobaculum orale* were more abundant the mild peri-implantitis (bone loss < 2mm) sites. Absolute abundance also presented with a severity-dependent relationship that higher species numbers were observed at sites with higher severity. Suppuration was associated with a lack of keratinized mucosa (75%), and > 6 mm bone loss (50%). The abundant species found in peri-implant pockets with suppuration is shown in Supplemental Figure 6. Bacterial abundance was significantly higher at the sites with suppuration (Figure 19). It is noteworthy that beta-diversity plot demonstrated two cases with suppuration without detectable radiographic bone loss was at the area with least microbial diversity; which indicated the phylogenetic (tree) distance was significantly distant from those with bone loss (Figure 20). A higher bacterial count was discovered at the sites in fully edentulous patients compared to the dentate patients, especially at peri-implantitis sites; however, bacterial diversity was found to be more diverse in those partial edentulous patients (Figure 21). In peri-implantitis sites in edentulous patients, *Dietziaceae, Mitochondria* family, and *Rickettsiales* were more frequently found (Figure 22). Finally, among those peri-implantitis sites with simultaneous presence of periodontitis on the remaining teeth, the microbial diversity within samples were found to be highest in the Stage 4 periodontitis when compared with Simpson's reciprocal index taking the richness and evenness of biodiversity into account (Figure 23). Although absolute abundance and beta-diversity seems to be similar among different severity of periodontitis, an unweighted PCoA revealed a clear clustering of samples, and the principal coordinates PC1 and PC2 together explained 60% of the variation between samples(Figure 24). The

abundant species standing out among different severity of periodontitis using LEfSe analysis were presented in Supplemental Figure 7.

Twelve biomarkers were analyzed using the Kruskal-Wallis test. The only significant difference found between healthy implant and peri-implantitis sites was IL-17 due to the difference of data variance (variance 14.2 vs 44 pg/ml). MMP-8 demonstrated a borderline significant difference between healthy implants and moderate to severe peri-implantitis (Table 24). MMP-8 further demonstrated a difference between healthy and peri-implantitis when the more severe cut-off points of bone loss were adopted. The higher the peri-implant bone loss, the higher the difference of MMP-8 between healthy and diseased implants (3145 vs 36.28.3 pg/ml in PI with bone loss> 4mm, p=0.05). PGE-2 showed the significant higher value in peri-implantitis until the bone loss cut-off point reached 3mm (147.2 vs 11.5.3 pg/ml, p=0.03). Lastly, when examining peri-implant inflammation (including mucositis) versus the healthy peri-implant sites, the positive association between biomarkers and peri-implant disease could be observed in the value of IL-6, IL-1β, IL-8, MMP-8, TIMP-2, and PGE2. IL-6 was the only biomarker that was not significant (Table 25).

Further ROC analysis explored the diagnostic value of these biomarkers. PGE-2 had significant diagnostic accuracy (AUC=57%) for a more severe form of peri-implantitis (> 4mm bone loss; Figure 25). MMP-8 had significant diagnostic accuracy (AUC=62%) for severe peri-implantitis (>4mm bone loss; Figure 26). The highest predictive ability for discerning healthy implant and peri-implant disease (Figure 27) was with IL-1β (AUC 70%), IL-8 (AUC 70%), MMP-8 (AUC 73%), TIMP-2 (AUC 74%), and PGE2 (AUC 69%). Lastly, chi-square test of the dichotomized prediction was performed to confirm the accuracy for moderate to severe peri-implantitis. The only significant predictor was IL-1β (p=0.02). For detecting peri-implant disease, a significant association of TIMP-2 and PGE2 was reported, while IL-1β and MMP-8 presented with borderline significance. (Table 26).

P. gingivalis, *T. forsythia*, and *F. nucleatum* were tested for association with disease manifestation using ROC analysis. All three species reached significance for the prediction of severe peri-implantitis. Bacterial counts were higher in the more severe form of peri-implantitis. AUC was 100% predictive of peri-implant inflammation (Figure 28) (Table 27). Dichotomized chi-square test was performed. *F. nucleatum* demonstrated significant sensitivity for predicting moderate to severe peri-implantitis. *T. forsythia* demonstrated borderline

significance. *P. gingivalis* demonstrated borderline significant prediction in peri-implant disease (Table 28). A combined prediction model was analyzed to predict peri-implant disease. *T. forsythia* and *F. nucleatum* demonstrated significant predictive ability combined with PICF biomarkers of MMP-8, IL-1β, and TIMP-2 with a higher predictive sensitivity and specificity compared to protein biomarkers alone. For differentiation of moderate to severe peri-implantitis, only *F. nucleatum* presented with significant sensitivity (approximately 70%) when combined with three protein biomarkers (Table 29).

Finally, GCF collected from the patients with periodontitis consistently demonstrated a significant difference between peri-implantitis and healthy sites with two cytokine biomarkers. OPG from periodontitis pockets (59.7±90.9 vs 26.6±51.6 pg/ml, p=0.01) and IL-6 from healthy sites on periodontitis patients (4.46±11.3 vs 1.56±0.86 pg/ml, p=0.04) was detected with higher levels at peri-implantitis group compared to the healthy implant group. Sensitivity and specificity of OPG and IL-6 to predict peri-implant disease was 48.9%, 56.5% and 17%, 79.2% respectively. These two biomarkers from healthy sites on periodontitis patients also demonstrated similar patterns associated with peri-implant diseases. TIMP-2 collected from periodontitis pockets was shown to be higher at peri-implant disease (PID) group than the healthy implant group (1420.5±583.4 vs 1082.2 ±458.4, p=0.04) with the sensitivity and specificity of 43.8% and 66.7%. Other GCF biomarkers didn't reveal any patterns related to peri-implant disease. For subjects with periodontitis, only IL-1β was higher in periodontitis pockets than in the healthy sites (164.7 vs 116.9 pg/ml).

Chapter V. Discussion

To the author's knowledge, this is one of the first investigations examining the association between peri-implantitis and cardiovascular diseases (CVD). In order to evaluate the potential risks low-grade chronic inflammation from peri-implantitis may pose to CVD, participants in the "Case" CVD group were rigorously recruited following the criteria that the dental implants were placed prior to CVD diagnosis (or cardiac event) being established. More importantly, the presence of peri-implantitis was evidenced radiographically before the CVD was diagnosed to ensure the temporal relationship between "exposure" and "disease". This case-controlled study revealed a higher prevalence of peri-implantitis (with detectable bone loss) in the CVD group (OR= 1.48,

p= 0.30). In order to discern the inflammatory burden of peri-implantitis and to decrease the probability of false positives, a more definitive criteria of moderate to severe peri-implantitis (BOP ± suppuration and radiographic bone loss ≥2mm) was implemented (Sanz et al. 2012; Derks et al. 2016). Moderate to severe peri-implantitis was significantly associated with the risk for CVD with an odds ratio of 2.18 (p=0.04). This significant difference persisted after adjusting for gender, and remained borderline significant at the stratum of 60 to 80 years of age (OR=2.5, p=0.06). Traditional risk factors for CVD were scrutinized in this investigation, The association between moderate to severe peri-implantitis and CVD after the adjustment for single confounders including smoking, alcohol, diabetes mellitus, BMI, metabolic syndrome, HDL level, hypercholesterolemia, or comorbidities remained significant. However, after controlling for all significant confounders, including age, smoking, hypertension, family history of heart attack, and the presence of periodontitis in the final multivariate logistic regression model, the significant association was no longer observed (adjusted OR= 1.36, p=0.54).

Atherosclerosis is an inflammatory process involving the host's immune mechanism interacting with other conventional risk factors to initiate, disseminate, and activate lesions throughout the cardiovascular system (Bentzon et al. 2014; Herrera et al. 2020). However, it cannot be fully explained by classical risk factors (Katz et al. 2001). Evidence has emerged demonstrating that low-grade chronic inflammation is not only associated with the increased prevalence of cardiovascular risk factors, but is also an independent risk factor for the development of CVD (Danesh et al. 2000; de Rooij et al. 2009; Koenig 2018). A number of chronic inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematous, ankylosing spondylitis, Sjögren's syndrome, polymyalgia rheumatica, inflammatory bowel disease, and psoriasis are associated with an increased risk of CVD (Haque et al. 2008; Coumbe et al. 2014; Ogdie et al. 2015; Agca et al. 2017; Eriksson et al. 2017; Feng et al. 2017; Ungprasert et al. 2017; Yong et al. 2018). Periodontitis, consistent with the rationale that chronic elevation in the systemic inflammatory burden was reported causally related to CVD development and its sequelae(Orlandi et al. 2020; Schenkein et al. 2020). Proposed mechanisms explaining the association between periodontitis and CVD include bacteremia and the associated systemic inflammatory cascades, including elevations in C-reactive protein and oxidative stress (Schenkein and Loos 2013; Herrera et al. 2020).

Peri-implantitis is considered a biological complication after dental implant placement (Heitz-Mayfield et al. 2014), exhibiting signs of inflammation and increased pocket depths over time. Peri-implantitis is a multifactorial disease triggered by inadequate treatment planning (including insufficient keratinized mucosa,

bone volume, implant proximity, 3-D implant position, and prosthetic design), local factors (history or ongoing compromised status of periodontitis, poor oral hygiene, and episodic periodontal maintenance), and host-factors (smoking, diabetes, immune susceptibility) (Berglundh et al. 2018; Schwarz et al. 2018). Given the similarities between peri-implantitis and periodontitis, the biological plausibility that chronic inflammation arisen from the continuously overwhelming bacterial insult propagates to distant tissues may be shared. The risk for atherothrombogenesis was identified in the higher levels of inflammation around dental implants with bone loss more than 2mm in the present study. Interestingly, the significant association with CVD seemed to emerge mainly in patients without diabetes, yet not in patients with metabolic syndrome in the current results. After controlling for significant confounding effects from age, hypertension, and family history, the probability for CVD remained higher but lost the statistical significance. It can be surmised that the impact from local inflammation is more evident among non-diabetic individuals, who were less likely under the influence of hyperglycemia with multiple comorbidities and increased risk for CVD(Leon and Maddox 2015). Instead, subjects with metabolic syndrome are inherently exposed to a cluster of risk factors for CVD. It is arguable that the local inflammation around dental implants had less influence on the potential propagation of inflammation compared to the pre-existing systemic inflammation burden originating from poor glycemic control, visceral obesity, metabolic syndrome, hypertension, lipid protein, and health behaviors (such as alcohol intake and smoking habits).

Inflammation and infection induce a variety of alterations in lipid metabolisms that may initially suppress inflammation or fight infection. However, chronic inflammation could contribute to the increased vascular inflammation and atherosclerosis (Khovidhunkit et al. 2004). The most common changes in lipid profiles related to the low-grade chronic inflammation are the decreases in HDL cholesterol and increases in triglycerides due to an increase of hepatic VLDL production and a decrease in the clearance of triglyceride-rich lipoproteins (Feingold and Grunfeld 2019). Dyslipidemia with consequent increases in pro-inflammatory lipid classes, along with common genetic susceptibility factors, leads to escalated inflammatory responses (Reyes et al. 2013; Schenkein and Loos 2013). Overall, the percentage of participants recognized as hypertriglyceridemia was higher in the non-CVD group compared to the CVD group (13.5% vs 6%). There were 51% participants in the non-CVD group with LDL-C levels above normal. On the contrary, only 22% of CVD group were found to be above normal levels. Nevertheless, the higher percentage (34%) of participants among the CVD group revealed low (suboptimal) HDL-C compared to 13.5% in the non-CVD group. Overall, the lipid profile of individuals in

the non-CVD group exhibited a predisposition to dyslipidemia which may partly account for the inflammatory profile among participants in the two groups and may impact the association between the two diseases. The difference of lipoproteins between the case and control groups may be explained by the overwhelming use of statin in the CVD group (60%) compared to the non-CVD group (28%). Statin (HMG-CoA reductase inhibitors) can effectively reduce the circulating levels of atherogenic LDL via regulation of LDL receptors (Nissen et al. 2005; Kinlay 2007). The clinical success has been confirmed by the meta-analysis of large randomized trials and has proven that atherosclerosis progression is attributed to the elevated LDL (Baigent et al. 2005; Baigent et al. 2010). Investigations have shown that statins are anti-inflammatory and are associated with reduced serum markers of inflammation (hsCRP), reduced T-cell and monocyte activation, and reduced blood clotting (Quist-Paulsen 2010). The effect of statins on HDL increase is modest and independent of LDL-C level (McTaggart and Jones 2008; Barter et al. 2010), which may explain the results we observed in the current dataset.

It was no surprise that the concentration of serum pro-inflammatory biomarkers in the present study including IL-1β, TNF-α and fibrinogen were higher in the CVD group independent of the local peri-implant inflammatory status. IL-6 and OPG also demonstrated a similar trend towards elevated biomarkers among participants with CVD. Interleukins mediating the signaling of leukocytes contribute profoundly to the atherosclerosis process (Dinarello 2011). IL-1β is strongly associated proatherogenic properties including the upregulation of endothelial adhesion and activation of macrophages and vascular cells (Clarke et al. 2010; Ait-Oufella et al. 2011). Notable effects of IL-1ß include the induction of prostaglandin production through induction of cyclooxygenase-2 (COX-2), the elaboration of nitric oxide, and activation of innate immune cells (Libby 2017). Not only does the increased IL-1β trigger a series of inflammatory reactions to promote bone resorption, IL-1β blockage has become the therapeutic strategy for autoinflammatory diseases such as T2DM, rheumatoid arthritis (RA), and gout (Cheng et al. 2020). Polymorphism of the IL-1 gene cluster is associated with severe periodontitis(Brodzikowska et al. 2019). Furthermore, IL-1RN gene polymorphism has been shown to be a risk factor for peri-implantitis (Laine et al. 2006).

Among cytokines, TNF-α and IL-6 are implicated in chronic inflammatory diseases. They are known for modulating immune reactions and acute response phase in inflammation (Le and Vilcek 1989) and secreted in response to stress hypoxia and tissue destruction which lead to cardiac cachexia after ingestion of apoptotic cells (Lleo et al. 2008). TNF-α is implicated as the principal pro-inflammatory cytokine from both animal (Csiszar et

al. 2007) and human (Moreau et al. 2013) models contributing to vascular dysfunction. More importantly, it has been shown that TNF-α upregulates the oxidative stress via increased production of reactive oxygen species, which has been linked to arterial stiffness in the aged population (Moreau et al. 2006) where increased adiposity-induced inflammation and comorbidities are more prevalent (Carter et al. 2018). IL-6 derived from T lymphocytes, macrophages, and adipocytes, together with IL-1 and TNF-α have been found to be the key players at the downstream of vascular inflammatory cascade of accelerating atherosclerosis (McInnes et al. 2015). Also, the risk of future myocardial infarction among healthy men has been shown most likely related to plaque instability (Ridker et al. 2000; Anderson et al. 2013). IL-6 and TNF-α have been reported to be consistently increased in patients with chronic inflammatory diseases such as rheumatoid arthritis (RA) since both play the central roles in the systemic inflammatory reactions (Hernández-Rodríguez et al. 2003; Sattar et al. 2003; Umare et al. 2014). These evidences coincided with the results of the current investigation that IL-6 and TNF- α demonstrate a fair predictive ability of CVD, as well as a dose-responsive association with the 10-year ASCVD risk.

Fibrinogen is inherently low in serum, since the coagulants are present mainly in plasma. Yet, the unified protocol of serum collection without the anticoagulants in all samples may allow for more standardized results, especially when multiplex immunoassay are analyzed (Tuck et al. 2009). The concentration of fibrinogen detected in the current investigation was approximately 60 mg/dl, which was much lower than the normal level in plasma (200-400 mg/dl). Still, by high-sensitivity ELISA array designed for serum samples, the significant difference of fibrinogen between CVD and non-CVD group was appreciated and showed a fair predictive ability of an AUC 65% in the ROC analysis. Elevated levels of fibrinogen in serum have been related to increased blood viscosity and thrombus formation; and a positive association between plasma fibrinogen and CVD, independent of other atherogenic factors and antithrombotics such as aspirin, has been reported (Kannel et al. 1987; Ma et al. 1999; Palmieri et al. 2003). In a meta-analysis of a large number of individuals, a moderate association has been found between fibrinogen levels and the risk of CHD, stroke, and other vascular mortality (Danesh et al. 2005). Recently, among the growing number of molecules for potential utility as CVD biomarkers, much attention has been centered on osteoprotegerin (OPG) and its ligands, including the receptor activator of nuclear factor kB ligand (RANKL) and TNF-related apoptosis-inducing ligand (TRAIL) (Hofbauer and Schoppet 2004). OPG, a regulatory protein for bone metabolism and vascular calcification, has been found to be associated with CVD pathophysiology (Bucay et al. 1998; Hofbauer et al. 2001) and cardiovascular

mortality and morbidity (Jono et al. 2002; Lieb et al. 2010). There is a strong evidence of correlation between the RANK-RANKL-OPG system and the atherosclerotic plaque calcification(Dhore et al. 2001; Schoppet et al. 2004). The immunological process of atherosclerosis has been shown to be related to increased expression of RANKL from T-cell that increases in leukocyte response and matrix degradation, leading to plaque destabilization (Shaker et al. 2010). OPG as a decoy receptor of RANKL may behave as an anti-inflammatory mediator and inhibit TRAIL-associated apoptosis in susceptible endothelial cells. The serum concentration of OPG in the current results corresponded to the ASCVD 10-year risk; which was in agreement with previous observations that it is a promising biomarker for ASCVD, regardless of conflicting data about the potential cardioprotective role(Özkalaycı et al. 2018). Lastly, serum hsCRP didn't show the significant difference and demonstrated an inverse relationship with the CVD group. The discussion of this finding is listed in the Supplement Table 2.

The evidences of biomarkers accumulated for peri-implant disease are mainly focused on PICF proteins rather than systemic markers in serum (Faot et al. 2015; Ghassib et al. 2019). In general, they are in concordance with the current results, that PGE2, IL-1β, IL-6, IL-8, OPG, TNF-α, MMP-8, VEGF, and TIMP-2 were in higher concentrations at inflammed peri-implant sites; but only PGE2, IL-1β MMP-8, and TIMP-2 reached the statistical significance. In the comparison between healthy implants and moderate to severe peri-implantitis, only MMP-8 reached a borderline significance (3387 vs 3104 pg/ml, p=0.09). To summarize, four PICF biomarkers (PGE2, IL-1β, MMP-8, and TIMP-2) were demonstrated in the current investigation to be strong predictors for the peri-implant inflammation. However, the ability to differentiate the peri-implantitis with mild bone loss was not strong enough to reach statistical significance. Only the severe form of peri-implantitis manifested with a significantly higher concentration of MMP-8. Overall, locally PICF biomarker did not demonstrate a capacity to differentiate between CVD and non-CVD group. Only TNF-α was predominantly higher in the CVD group, and coincided with the local peri-implant inflammation. TNF-α has been reported as the most common cytokine isolated from patients with peri-implantitis (Konttinen et al. 2006). Interestingly, it has been correlated with severe peri-implantitis and reduced significantly comparable to the control sites 3 months after the mechanical anti-infective therapies (de Mendonça et al. 2009; Duarte et al. 2009b). TNF-α underlines the real-time manifestation of inflammation; and when the fibroblasts in the chronic peri-implantitis granulation tissue are unable to switch off the pro-inflammatory pathway, both migration and retention of

leukocytes may occur continuously within the sites in a self-feeding loop (Bordin et al. 2009), which may explain why the results in current study coincided with the systemic inflammatory burden.

The correlation of PGE2 with clinical manifestations of periodontal and peri-implant inflammation has been shown in cross-sectional studies (Heasman et al. 1998; Preshaw and Heasman 2002; Yalçn et al. 2005). It is in agreement with the current investigation that predictability for peri-implant inflammation was with AUC=69% and significantly higher in the severe peri-implantitis group. This implied that the severe peri-implantitis sites have higher vasodilation, increased vascular permeability, activation of osteoclasts, and mediation of bone resorption (Offenbacher et al. 1993). This is in agreement with the report that elevated PGE2 and MMP-8 are correlated with the peri-implant mucosa inflammation and pocket depth increase, especially MMP-8 which appears to be an early signal of peri-implant inflammation (Basegmez et al. 2012). MMPs have been collectively related to the degradation of the extracellular matrix and basement membrane during tissue destruction and remodeling (Ryan and Golub 2000) which manifests in the cases in peri-implant disease. In addition, MMP-8 as a neutrophil collagenase, has been shown to be triggered by potent periodontopathogenic bacteria and their virulence factors (Sorsa et al. 2016). Thus, elevated MMP-8 has been shown significantly associated with soft and hard tissue destruction such as in periodontitis and peri-implant disease (Teronen et al. 1997; Nomura et al. 1998; Nomura et al. 2000; Xu et al. 2008). Furthermore, the concentration of MMP-8 is correlated to the severity of peri-implant bone loss (Ma et al. 2000; Kivelä-Rajamäki et al. 2003). The strong predictive ability of MMP-8 as an adjunctive diagnostic tool has been reviewed in the literature (Al-Majid et al. 2018; Ghassib et al. 2019). This is consistent with the current findings that the predictability was 62% for severe peri-implantitis (bone loss >4mm) and 73% for peri-implant inflammation, respectively. It was noteworthy that it demonstrated a significant sensitivity to predict moderate to severe peri-implantitis when combined with *T. forsythia* and *F. nucleatum* and the power was higher compared to the prediction model restricted to biomarker or microbial profile alone. Tissue inhibitor of metalloproteinases-2 (TIMP-2) is known for its ability to inhibit protease activity and suppression of endothelial cell proliferation (Stetler-Stevenson 2008). Its anti-inflammatory and anti-catabolic features have been shown in a comparative study. After non-surgical treatment of peri-implantitis sites, increased expression of TIMP-2 is detected (Ghighi et al. 2018). A disruption of the MMP-TIMP balance may lead to a pathological process of losing ECM, such as arthritis, atherosclerosis, and periodontitis. An elevated expression of TIMP-2 has been reported in chronic periodontitis and is elevated even more when concomitant diabetes is present (Shin et al. 2010). It has been reported in a cross-sectional study that

TIMP-2 behaves as an effective predictor of peri-implantitis (OR= 4.4). When the diagnostic tool is combined with the microbial profile of *T. denticola*, the power of prediction increases (Wang et al. 2016). However, such trend was not appreciated in the current results. Additional discussion pertaining to the non-significant findings of OPG, IL-6, IL-8, and VEGF is listed in the Supplemental Table 3.

Evidence suggests that oral bacteria can enter the blood stream and cause bacteremia either following daily toothbrushing, chewing or professional interventions (periodontal probing, scaling, or surgical extraction) (Balejo et al. 2017) and is fundamentally associated with the gingival inflammation (Tomás et al. 2012). Furthermore, periopathogenic pathogens have been identified in the atherosclerotic plaques (Mahendra et al. 2013; Armingohar et al. 2014). Viable *P. gingivalis* and *A. actinomycetemcomitans* have been discovered in atherothrombotic lesions within atheroma samples(Kozarov et al. 2005; Rafferty et al. 2011). Particularly, *P. gingivalis* has been shown to accelerate atherosclerosis in murine models and induce aortic and coronary lesions after bacteremia in normocholesterolemic pigs (Schenkein and Loos 2013). *In vitro* evidence also supports the importance of the fimbriae of *P. gingivalis* to adhere and enter human coronary endothelia cells and to promote atherothrombotic lesions (Bélanger et al. 2012; Yang et al. 2014). *P. gingivalis, A. actinomycetemcomitans, T. denticola,* and *T. forsythia* in subgingival plaque samples have been reported to be associated with carotid intima-media thickening in the adjusted analysis of a large population study (Desvarieux et al. 2005). Recently, a polymicrobial experimental infection (*P. gingivalis, T. forsythia, T. denticola, and F. nucleatum*) has been shown to induce the aortic toll-like receptors (TLRs) and inflammasome signaling and enhance the oxidative stress reaction within the aortic endothelial cells (Velsko et al. 2014; Chukkapalli et al. 2015). Commensal pathogen, *F. nucleatum,* can turn to periopathogenic which has been linked to acceleration of aortic atherosclerosis in an apolipoprotein E $(ApoE)^{null}$ hyperlipidemic mice model (Han 2015; Velsko et al. 2015). It has been implicated in the association with CVD (Han and Wang 2013). Additionally, it has been detected in ruptured cerebral aneurysm (Ford et al. 2006; Figuero et al. 2011; Pyysalo et al. 2013) and related to the severity of periodontitis (Elkaim et al. 2008).

The microbial DNA sequencing profile established in the current investigation demonstrated the relative abundance of *P. gingivalis* was higher in the CVD group (1.7% vs 1%). Subsequent PCR analysis also showed absolute bacterial counts of *P. gingivalis* was higher in the CVD group (5.3E+07 vs. 3.9E+07). The same tendency was found favoring the periodontitis subgroup ($p>0.05$). It can be said the bacterial counts of *P.*

gingivalis was in agreement with the previous findings. Another interesting finding was that higher CRP detection in peri-implant sulci was associated with the higher detection frequency of *P. gingivalis*, which may be correlated to the intricate link between inflammatory challenge by local bacterial insult and the cross-talk with the systemic inflammatory mediators. Secondly, our results showed peri-implant pockets appeared to harbor higher concentrations of *F. nucleatum* in a dose-responsive fashion associated with the severity of peri-implantitis. Although the magnitude failed to fully correspond to the severity of the disease, higher counts of *P. gingivalis* and *T. forsythia* were found at peri-implant inflamed sites compared to the absolute healthy peri-implant sites. *T. forsythia* has been reported as the most frequently detected species with the increased magnitude of peri-implant disease (Charalampakis et al. 2012). On the other hand, *F. nucleatum* and *P. gingivalis* are correlated to the implant sites with the deepest probing depth (Renvert et al. 2007). Our observation was consistent with a variety of reports in peri-implantitis with regard to the predominance of these three species and a more divergent profile of the microbial community compared to chronic periodontitis (Mombelli 2002; Shibli et al. 2008; Pérez-Chaparro et al. 2016; de Waal et al. 2017; Lafaurie et al. 2017; Schwarz et al. 2018; Costa et al. 2019; Sahrmann et al. 2020). Peri-implant PD > 5mm with concomitant BOP, along with a full-mouth plaque scores> 20%, are generally considered as major risk factors for the peri-implantitis (Heitz-Mayfield et al. 2014; Heitz-Mayfield et al. 2020). Our results supported the observations that predominantly anaerobic microorganisms at peri-implantitis niche are associated with deeper pockets and severe bone loss (Augthun and Conrads 1997; Cortelli et al. 2013). *T. forsythia* especially stands out in the lesions with bone loss > 4mm. Partial edentulous individuals have been reported to potentially harbor more pathogens compared to those fully edentulous subjects in a recent systemic review (de Waal et al. 2014). Data from this study also indicated that partial edentulous individuals were found to harbor a more divergent composition of the microbial community. However, *P. gingivalis, T. forsythia, and F. nucleatum* were found to be higher in the edentulous subgroup ($p<0.01$ in the difference of *T. forsythia*). Prudent interpretation should be advised since our sample size for total edentulous patients was limited (n=10).

Due to the complexity of the microbial community of the samples in the current investigation, it was difficult to conclude a certain pattern of predominance of species between case and control groups. Yet, an interesting finding of this study was that *Haemophilus parainfluenzae* was detected more frequently in the CVD group (2.7% vs 0.6%). *Haemophilus parainfluenzae*, one of the HACEK organisms commensal to the human oral cavity, is a Gram (-) opportunistic pathogen that has been associated with infective endocarditis, bronchitis, and

pneumonia (Duzenli et al. 2017). Another opportunistic pathogen found in the current observation which is strongly associated with infective endocarditis (IE), was *S. aureus*. It is the most common pathogen entering the blood stream and has accounted for 31.4% of definite IE reported in a large prospective-cohort study most commonly found related to health care (39.1%) (Fowler et al. 2005). It has been reported as nosocomial infections in IV drug users and prosthetic valve recipients (Miro et al. 2005). New at risk categories for *S. aureus* infections include increased patient age, elevated drug resistance, intensive treatment such as renal hemodialysis, immuno-suppression, long-term indwelling central venous catheter, and the application of modern cardiac device implants (Hoerr et al. 2018). It has been viewed as one strongly relative pathogen in the chronic infection around dental implants (Mombelli and Décaillet 2011; Persson and Renvert 2014) and has been shown to colonize implants early after placement (Fürst et al. 2007) and persists long-term (Salvi et al. 2008). It has been associated with deeper peri-implant pockets or suppurative failing implants in some reports(Leonhardt et al. 1999; Kronström et al. 2001; Albertini et al. 2015). This is consistent with observations in this study. The susceptibility of *S. aureus* infection is not clear, since there is a lack of humoral immunity factors involved in osseointegration (Kronström et al. 2000; Albertini et al. 2015) and the affinity of *S. aureus* to titanium (Harris et al. 2007). There is a disparity in the reports of *S. aureus* in peri-implantitis sulci by DNA-DNA checkboard (Renvert et al. 2007; Renvert et al. 2008; Salvi et al. 2008; Persson and Renvert 2014) or culture techniques (Canullo et al. 2016; de Waal et al. 2017). However, there are only few studies that identify *S. aureus* in the peri-implantitis sites using the RT-qPCR. The higher detection frequency reported by Zhuang et al. compared to the current study may be attributed to the primer design targeted to a different strain (Mu3 as opposed to Sau31 strain) and the study design differences (within-same-subject comparison) (Zhuang et al. 2016). On the contrary, the result from Canullo et al. using qPCR is in line with our results showing *S. aureus* was detected in very low bacterial counts (Canullo et al. 2015).

In conclusion, it is reasonable to extrapolate the same biological mechanisms of chronic periodontitis to the chronic peri-implantitis which shared some similarities of low-grade chronic inflammation at gingiva/mucosa tissue, including the soft tissue reactions to plaque formation, such as the B and T-cell-dominated inflammatory cell infiltrates and tissue breakdown (Sanz et al. 1991; Zitzmann et al. 2001; Konttinen et al. 2006; Salvi et al. 2012), and the similar microbiologic niche harboring key common anaerobic periodontopathogens, such as *P. gingivalis, F. nucleatum* and *T. forsythia (Mombelli 2002; Koyanagi et al. 2013; Persson and Renvert 2014; de Waal et al. 2017; Lafaurie et al. 2017; Schwarz et al. 2018)*. Overall, our results supported the biologic

plausibility that low-grade chronic inflammation around diseased dental implants, especially when the disease severity and tissue destruction increased, may have an implicit role in the manifestation and progression of atherosclerosis and subsequent cardiovascular sequelae. The results in the current investigation showed an increasing amount of TNF-α in the CVD group that coincided with the local peri-implant inflammation. This suggests that the low-grade chronic inflammation may be the common denominator between CVD and peri-implantitis and among the shared risk factors/indicator for these two diseases.

The limitations of current investigation included the lack of ability to establish the causality of these diseases in this case-control design, cross-sectional study. Secondly, confounding bias cannot be ruled out because the case of CVD was established regardless of the disease severity and the time elapse since the diagnosis. Albeit the association between peri-implantitis and CVD was lessened to a non-significant relationship after controlling for multiple cofounders, the result of current report might present the influence of a risk indicators of peri-implant disease for the CVD on the sub-elements of disease manifestation. It emphasizes the need of both clinical and radiographic evaluation of dental implants on a regular basis to reduce the concomitant risks for CVD and also sheds light on the need for future research to understand the complex inter-relationship between local inflammation around dental implants and the systemic inflammatory burden to cardiovascular risk.

Chapter VI. Future Research Direction

The result of current investigation revealed an association of CVD to a more severe manifestation of peri-implantitis, and the escalated level of pro-inflammatory cytokines observed in our findings supported the hypothesis that consistently chronic peri-implant inflammation may pose a higher risk for the development of CVD. Such association seems to augment as the severity of disease increased; however, the association was not found in the severe peri-implantitis which may related to the limited sample size in that stratum. Therefore, future studies with a larger sample size were warranted; moreover, to control for possible patient-related confounders with age- and gender-matched design and relevant implant-related factors with same settings such as tissue-level or bone-level implants would be suggested for the future research direction.

The relationship between the prevalence of *P. gingivalis* around diseased implants and CVD also called for more future human studies to illuminate the potential causal relationship between the peri-implant disease and CVD. Lastly, as the case-control design may provide a higher power of evidence compared to the cross-sectional study to establish that peri-implantitis was a risk indicator for the cardiovascular disease; however, future cohort longitudinal studies were warranted to explore the possibility that chronic inflammation around dental implants may be a true risk factor for the cardiovascular disease.

Chapter VII. Conclusion

The present study assessed the odds of peri-implantitis as a possible risk indicator for the cardiovascular disease. The statistical analyses indicated that the association between peri-implantitis and cardiovascular disease was strongly influenced by the selection of threshold of bone loss defining disease severity. The moderate to severe form of peri-implantitis was significantly associated with cardiovascular disease (odds ratio 2.18). Although it remained borderline significant association among individuals between 60 to 80 years of age after controlling for gender, but a null association was observed after adjustment for multiple significant confounding factors.

The low-grade chronic inflammation around diseased dental implants, especially when the disease severity and tissue destruction increased, may be a factor relating these two diseases. The result of the current investigation sheds light in understanding the relationship between peri-implant chronic inflammation and cardiovascular diseases. Future study should include a prospective clinical trial that follows patients with peri-implantitis for a period of time and see if the disease eventually leads to the development of cardiovascular disease.

Chapter VIII. Tables and Figures

Table. 1 Epidemiologic evidence of the relationship between cardiovascular disease and peri-implantitis or implant failure

Study	Types of Study	Study Sample	Cardiovascular disease	Case definition of peri-implantitis	Results
Renvert et al. 2014	Case-control	• 172 subjects with peri-implantitis • 98 subjects with healthy implants	History of CVD (acute coronary syndrome, stroke, aneurysm, heart arrhythmia, ischemia, heart valve disease, atherosclerosis, and hypertension with medication)	• BOP±suppuration • Bone loss ≥ 2 mm	A history of CVD was found in 27.3% of individuals with peri-implantitis (OR=87); 3% in the healthy implant/mucositis group
Roos-Jansaker et al 2006	Cohort	294 subjects (999 implants)	General disease (coronary heart disease, diabetes, osteoporosis)	• BOP±suppuration • Bone loss ≥ 1.8 mm (3 threads)	No significant association
Koldsland et al 2011	Cross-sectional	109 subjects (999 implants)	Systemic disease with particular focus on cardiovascular disease (lung disease/allergy, DM, RA, osteoporosis, and immune deficiency)	• BOP±suppuration • Detectable peri-implantitis: bone loss >0.4 mm • Overt peri-implantitis: bone loss ≥ 2 mm	No significant association
Casado et al 2013	Cross-sectional	215 subjects (754 implants)	Systemic disease (cardiovascular disease, hypertension, asthma)	>1 mm within first year, and > 0.2 mm for year	No significant association
Dalago et al 2017	Cross-sectional	183 subjects (916 implants)	Heart disorders	• BOP±suppuration • Bone loss > 2 mm	Associated in the univariate level(13.1% vs 6.9 %); not significant after multivariate level analysis
Frisch et al 2014	Retrospective	236 subjects (540 implants)	Cardiovascular disease	• BOP±suppuration • Bone loss ≥ 2 mm	No association
Lachmann et al 2013	Cross-sectional	74 subjects (236 implants)	Cardiovascular disease (hypertension, cardiac infarction, arrhythmia, not specified)	• BOP±suppuration • Changes of bone level (not specified)	• CVD was the most prominent co-morbidity (27%) of peri-implantitis • CVD was significantly associated with the prevalence and concentration of *P. intermedia*
Lee et al 2010	Cohort	35 subjects (118 implants) (more than 70 y/o)	Systemic disease (hypertension, DM, heart disease, kidney disease, thyroid disease, etc.)	• BOP±suppuration • Progressive bone loss	Peri-implant bone loss is associated with CVD (p>0.05)
Alsaaadi et al 2016	Retrospective	2004 subjects (6946 implants)	Hypertension, ischemic cardiac problems	Implant failure, up to abutment connection	Not associated with early implant failure

Shimizu et al 2012	Cohort	70 subjects with diabetes (352 implants)	Cardiovascular disease	Implant survival	Implant survival not associated with co-existing CVD and DM
Austin et al 2015	Retrospective	44,415 subjects (6946 implants)	Heart disease (cardiovascular pathologies including hypertension)	Implant failure	Heart disease was significantly associated with implant failure
Burrowes et al 1992	Retrospective	13 subjects with high risk of infective endocarditis (IE) (57 implants)	Aortic valve replacement, mitral valve replacement, mitral valvuloplasty, tetralogy of Fallot, and history of IE	–	Within the follow-up years (2-year), no IE was reported
Neves et al 2018	Retrospective	721 subjects (3998 implants)	Cardiac disease	Implant failure	Cardiac disease was correlated with a higher number of implant failure ($p>0.05$)
Wu et al 2016	Retrospective	728 subjects (1449 implants)	Anti-hypertensive drugs (142 user/586 non-users)	Implant survival	Anti-hypertensive drugs were associated with higher implant survival rate

Table 2. Demographic variables and CVD risk factors

Variable		non-CVD (n = 46) n [%]	CVD (n =82) n [%]	P Value
Age [mean ± SD (range)]		69.3 ± 10.1 (43-86)	75.0± 8.3 (50-91)	**0.004** §
Age	40-49 y	3 (6.5)	0 (0)	**<0.01**
	50-59 y	2 (4.3)	5 (6.1)	
	60-69 y	16 (34.8)	12 (14.6)	
	70-79 y	17 (37.0)	41 (50)	
	≥ 80 y	8 (17.4)	24 (29.3)	
Gender				0.08
	Female	23 (50)	28 (34.1)	
	Male	23 (50)	54 (65.9)	
Ethnicity				0.08
	White	36 (78.3)	74 (90.2)	
	Black	3 (6.5)	3 (3.7)	
	Hispanic	3 (6.5)	0 (0)	
	Asian or other	4 (8.7)	5(6.1)	
Smoking				**0.03**
	Never-smoker	28 (60.9)	30 (36.6)	
	Current smoker	2 (4.3)	4 (4.9)	
	Ex-smoker	16 (34.8)	47 (57.3)	
	<=5 years	1 (6.3)	3 (6.3)	0.52
	5-10 years	2 (12.5)	3 (6.3)	
	10-15 years	2 (12.5)	2 (4.2)	
	>15 years	11 (68.8)	40 (85.1)	
Alcohol				0.59
	Non-drinker	14(30.4)	31 (37.8)	

Social drinker	15 (32.6)	17 (20.7)	
<7 units /week	12 (26.1)	21 (25.6)	
7-14 units /week	4 (8.7)	9 (11)	
>14 units /week	0 (0)	1 (1.2)	
BMI			0.85
Normal	17 (37)	23 (28)	
Overweight	15 (32.6)	30 (36.6)	
Obese	12 (26.1)	21 (25.6)	
Severe Obese	2 (4.3)	3 (3.7)	
Metabolic Syndrome	20 (43.5)	45 (54.9)	0.22
Diabetes Mellitus¶	8 (17.4)	24 (29.3)	0.14
Hypertension¶	18 (39.1)	49 (59.8)	**0.03**
High Cholesterol¶	16 (34.8)	38 (46.3)	0.20
Osteoporosis or bone-related disease¶	7 (15.2)	15 (18.3)	0.64
Rheumatoid arthritis¶	3 (6.5)	6 (7.3)	0.87
Co-morbidity			
1 co-morbidity	14 (30.4)	27 (32.9)	0.77
2 co-morbidity	10 (21.7)	17 (20.7)	0.89
3 co-morbidity	9 (19.6)	25 (30.5)	0.18
Family Health History			
Heart Attack	17 (37)	48 (58.5)	**0.01**
Stroke	21 (45.7)	29 (35.4)	0.33
Hypertension	25 (54.3)	48 (58.5)	0.46
Diabetes	18 (39.1)	40 (48.8)	0.27
Cancer	28 (60.9)	52 (63.4)	0.62
Medications			
Hypoglycemics	5 (10.9)	20 (24.4)	
Aspirin	11 (23.9)	37 (45.1)	
Anticoagulant	2 (4.3)	28 (34.1)	
Statin	13 (28.3)	49 (59.8)	
Angiotensin II receptor blocker	8 (17.4)	16 (19.5)	
ACE inhibitor	8 (17.4)	16 (19.5)	
Calcium channel blocker	4 (8.7)	15 (18.3)	
Beta-blocker	11 (23.9)	33 (40.2)	
Diuretics	7 (15.2)	24 (29.3)	

* P-value in bold indicated the significance ($p< 0.05$)

§ p value obtained from *t*- test without tithe assumption of equal variance; other p-values were obtained from chi-square test

¶ Patient-self-reported history of disease

Data may be missing in some individuals

Table 3. CVD categories within CVD group

Category	n [%]
Coronary Heart Disease	24 (29.3)
Cerebrovascular Disease	12 (14.6)
Peripheral Artery Disease	6 (7.3)

Rhythm Disorders	23 (28.0)
Pacemaker	12 (14.6)
Valvular Disease	6 (7.3)
Subclinical Atherosclerosis	2 (2.4)
Thoracic or Abdominal Aortic Aneurysm	2 (2.4)
Cardiomyopathy and Heart Failure	7 (8.5)

Table 4. Lipid profiles and Classification of atherosclerotic cardiovascular disease (ASCVD) risk assessment and cardiometabolic disease

Variable	non-CVD (n = 46) n [%]	CVD (n =82) n [%]	P Value
Fasting Glucose Level			0.28
Normal (<100 mg/dl)	14 (35.8)	14 (21.9)	
Prediabetes (100~125 mg/dl)	22 (55)	37 (57.8)	
Well-Controlled DM (126~153 mg/dl)	3 (7.5)	12 (18.8)	
Moderate Controlled (154~183 mg/dl)	1 (2.5)	1 (1.6)	
Total Cholesterol Type			0.07
Normal	28 (75.5)	61 (91)	
Borderline	8 (21.6)	6 (9)	
High	1 (2.7)	0 (0)	
Triglycerides Type			0.25
Normal	32 (86.5)	64 (94.1)	
Borderline	4 (10.8)	4 (5.9)	
High	1 (2.7)	0 (0)	
Triglycerides Severity			0.19
Normal	32 (86.5)	63 (94)	
Hypertriglyceridemia	5 (13.5)	4 (6)	
VLDL Type			0.32
<30	32 (86.5)	62 (92.5)	
>30 Elevated	5 (13.5)	5 (7.5)	
HDL Type			0.07
Low	5 (13.5)	23 (34.3)	
Borderline	19 (51.4)	27 (40.3)	
High (Optimal)	13 (35.1)	17 (25.4)	
cLDL Type			**<0.01**
Normal	18 (48.6)	58 (87.9)	
Above Normal	14 (37.8)	7 (10.6)	
Borderline High	4 (10.8)	1 (1.5)	
High	1 (2.7)	0 (0)	

cLDL Severity			**<0.01**
Normal	18 (48.6)	58 (86.6)	
Borderline	18 (48.6)	9 (13.4)	
Hypercholesterolemia	1 (2.7)	0 (0)	
Chol/HDL Ratio			0.08
Normal	33 (89.2)	66 (98.5)	
Borderline	2 (5.4)	1 (1.5)	
Risk	2 (5.4)	0 (0)	
LDL/HDL Ratio			**0.02**
Normal	21 (91.3)	61 (100)	
Risk	2 (8.7)	0 (0)	
ASCVD Risk Type			**<0.01**
Low	6 (16.2)	2 (3)	
Borderline	3 (8.10)	4 (6)	
Intermediate	16 (43.2)	15 (22.4)	
High	12 (32.4)	46 (68.7)	
Cardiometabolic Stage			**<0.01**
1	13 (30.2)	0 (0)	
2	11 (25.6)	0 (0)	
3	12 (27.9)	0 (0)	
4	7 (16.3)	82 (100)	

* P-value in bold indicated the significance in the chi-square test ($p< 0.05$)
Data may be missing in some individuals

Table 5. Prevalence of peri-implant disease and implant-related characteristics

Variable	non-CVD (n = 46)			CVD (n =82)		
	n [%]	PPD 4-6 mm, %	PPD >7 mm, %	n [%]	PPD 4-6 mm, %	PPD >7 mm, %
Peri-implant healthy	4 (8.7)	75	0	8 (9.8)	100	0
Peri-implant mucositis	16 (34.8)	75	0	21 (25.6)	76.2	9.5
Peri-implant: bone loss beyond initial physiological bone remodeling						
> detectable bone loss	26 (56.5)	84.6	15.4	53 (64.6)	77.8	18.9
Moderate to severe (≥2 mm bone loss)	14 (30.4)*	71.4	28.6	40 (48.8)*	75	25
Severe (>4 mm bone loss)	8 (17.4)	62.5	37.5	14 (17.1)	50	50
BOP						
<33%	7 (15.2)			10 (12.2)		
33-66%	21 (45.7)			28 (34.1)		
>66%	18 (39.1)			44 (53.7)		
Suppuration	7 (15.2)			7 (8.5)		
Implant prosthesis						
Fixed prosthesis	45 (97.8)			1 (2.2)		
Overdentures	70 (85.4)			12 (14.6)		

* indicated the significant difference between two groups in the chi-square test ($p< 0.05$)

Variable	non-CVD (n = 45) n [%]	CVD (n =72) n [%]	Odds ratio	P Value
Periodontal health	23 (51.1)	17 (23.6)	3.4	**<0.01**
Chronic periodontitis	22 (48.9)	55 (76.4)		
Severity				**< 0.01**
Stage 1	10 (41.7)	3 (4.5)		
Stage 2	7 (29.2)	52 (78.8)		
Stage 3	2 (8.3)	3 (4.5)		
Stage 4	5 (20.8)	8 912.1)		
Extent				**0.04**
Localized	12 (54.5)	26 (29.1)	2.9	
Generalized	10 (45.5)	39 (70.9)		
Grade*				**0.01**
Grade A	7 (31.8)	3 (5.4)		
Grade B	12 (54.5)	48 (87.3)		
Grade C	3 (13.6)	4 (7.3)		
Grade¶ (combined hsCRP)				0.16
Grade A	5 (12.5)	2 (2.9)		
Grade B	9 (19.6)	18 (25.7)		
Grade C	26 (56.5)	50 (71.4)		
Teeth loss number				
< 5 teeth	23 (51.1)	25 (30.5)		
5-10 teeth	18 (40)	28 (34.1)		**<0.01**
> 10 teeth	4 (8.9)	29 (35.4)		
Fully Edentulism	1 (2.2)	10 (12.2)		**0.05**
Maintenance frequency				
Episodic	23 (51.1)	46 (59.7)	0.71	0.23
Regular	22 (48.9)	31 (40.3)		

Table 6. Periodontal health variables

P-value in bold indicated the significance in the chi-square test ($p< 0.05$)
* Based on the Classification of 2017 World Workshop
¶ Combined the consideration of hsCRP

Table 7. Age-stratified analyses for the prediction of CVD

¶ p-value in bold indicated statistically significance (p< 0.05)
§ p-value indicated borderline significance
* adjusted for gender, smoking, hypertension, and family history of heart attack

Variable	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)
		Model 1	Model 2	Model 3
Peri-implantitis				
Detectable bone loss	1.48 (0.71 to 3.11)			
Bone loss≥ 2mm	**2.18 (1.02 to 4.67)**	1.64 (0.58 to 4.7)	1.67 (0.54 to 5.15)	1.14 (0.35 to 3.75)
Bone loss> 3mm	1.39 (0.63 to 3.05)			
Bone loss> 4mm	0.98 (0.38 to 2.54)			
Age		**1.05 (1.0 to 1.11)**	0.96 (0.87 to 1.06)	**1.1 (1.03 to 1.14)**
Hypertension		0.84 (0.29 to 2.44)	0.71 (0.23 to 2.2)	0.64 (0.2 to 2.12)
Family history of heart attack		2.35 (0.84 to 6.57)	2.66 (0.90 to 7.88)	2.39 (0.8 to 7.12)
Smoking				
Non-smoker		**referent**	**referent**	**referent**
Ever-smoker		**5.19 (1.71 to 15.7)**	**5.76 (1.83 to 18.2)**	**3.81 (1.12 to 13.0)**
cLDL level		**0.12 (0.04 to 0.38)**	**0.11 (0.03 to 0.39)**	**0.12 (0.03 to 0.45)**
ASCVD risk type				
Low			referent	
Borderline			5.9 (0.24 to 149.6)	
Intermediate			4.55 (0.24 to 84.0)	
High			33.9 (0.93 to 1385.5)	
Chronic Periodontitis				**3.59 (0.99 to 13.0)**

Moderate to severe peri-implantitis= radiographic bone loss≥2 mm and BOP± suppuration

Table 8. Multivariate logistic regression model for the prediction of CVD

Odds ratio (OR) in bold denotes the significance in the multivariate logistic regression (p< 0.05)

Table 9. Serum biomarker profiles between case (CVD) and control (non-CVD)

Categories	Model Moderate to severe peri-implantitis only adjusted for gender		Model Moderate to severe peri-implantitis*	
	OR (95% CI)	p-value	OR (95% CI)	p-value
All population (range 43-91 y)	2.3 (1.1 to 5.0)	**0.04**¶	2.0 (0.8 to 4.6)	0.12
40-60 y	2.7 (0.12 to 59.1)	0.54	1.0	0.49
60-80 y	2.5 (0.9 to 6.5)	**0.06** §	1.9 (0.6 to 5.6)	0.24
>80 y	2.1 (0.4 to 11.4)	0.38	3.4 (0.5 to 24.5)	0.24

SD: standard deviation
*denotes the statistically significant difference (P<0.05) from Kruskal-Wallis test

Table 10. Stratified analysis of Serum CRP

Stratified subgroup		mean±SD (mg/L)	median	P-value
Statin therapy (No)	non-CVD	8.7±3.9	9.9	0.09
	CVD	6.7±3.8	6.1	
Statin therapy (Yes)	non-CVD	5.3±4.2	4.7	0.51
	CVD	6.2±3.4	6.6	
Normal LDL	non-CVD	6.3±4.0	5.8	0.93
	CVD	6.3±3.6	6.4	
Borderline to high LDL	non-CVD	8.8±4.0	10.1	0.47
	CVD	7.5±3.5	6.7	

SD: standard deviation
*denotes the statistically significant difference (P<0.05) from Kruskal-Wallis test

Table 11. Diagnostic ability of serum biomarkers for detecting CVD

	AUC	95% CI	*p-value*
Serum IL-1β	0.63	0.50-0.76	0.08
Serum IL-6	0.64	0.50-0.79	0.06
Serum MMP-8	0.53	0.43-0.43	0.31
Serum OPG	0.58	0.40-0.73	0.31
Serum TNFa	0.67	0.56-0.78	**0.02***
Serum hsCRP	0.44	0.51-0.79	0.41
Serum Fibrinogen	0.65	0.51-0.79	**0.04***

Biomarker	non-CVD mean(SD)	non-CVD Median	CVD mean (SD)	CVD Median	p-value
IL-1β (pg/ml)	58.4± 103.0	17.9	97.0± 136.8	33.6	**0.02***
IL-6 (pg/ml)	191.3± 362.4	6.1	267.3± 570.6	27	0.09
MMP-8 (pg/ml)	33.2± 101.1	13.2	29.1± 54.5	13.2	0.66
OPG (pg/ml)	1044.5± 846.6	758.2	1287.8± 925.1	968.4	0.09
TNF-α (pg/ml)	56.5± 152.1	6.9	104.7± 161.7	31.6	**<0.01***
CRP (mg/L)	7.7± 4.2	9.2	6.4± 3.5	6.5	0.11
Fibrinogen (mg/dl)	62.3± 35.7	50.9	86.2 ± 61.5	70.0	**0.03***

AUC: Area under the curve; CI: confidence interval

Table 12. Serum diagnostic ability of serum biomarkers

Biomarker	Group	Threshold	Marker level		Sensitivity	Specificity	OR	95% CI	p-value
			High	Low					
IL-1β	CVD	19.3(pg/ml)	41	29	58.6%	63.2%	2.4	1.1-5.4	**0.03***
	non-CVD		14	24					
IL-6	CVD	20.7(pg/ml)	38	16	50.3%	57.9%	1.6	0.7-3.6	0.23
	non-CVD		16	22					
MMP-8	CVD	13.2(pg/ml)	15	56	21.1%	35.6%	1.2	0.4-3.2	0.74
	non-CVD		7	31					
OPG	CVD	897(pg/ml)	49	8	86%	45.2%	5.0	1.8-14.1	**<0.01***
	non-CVD		17	14					
TNF-α	CVD	14.6(pg/ml)	42	28	60.0%	65.8%	2.9	1.3-6.6	**0.01***
	non-CVD		13	25					
hsCRP	CVD	6.8(mg/L)	28	34	45.2%	43.3%	0.6	0.3-1.6	0.6
	non-CVD		17	12					
Fibrinogen	CVD	64.8(mg/dl)	38	31	55.1%	58.3%	1.7	0.8-3.9	0.19
	non-CVD		15	21					

ǂ denotes the significant difference between groups from χ^2 test ($P < 0.05$)

Figure 1. Receiver operating characteristic (ROC) curve for the diagnostic ability of serum biomarkers for detecting CVD

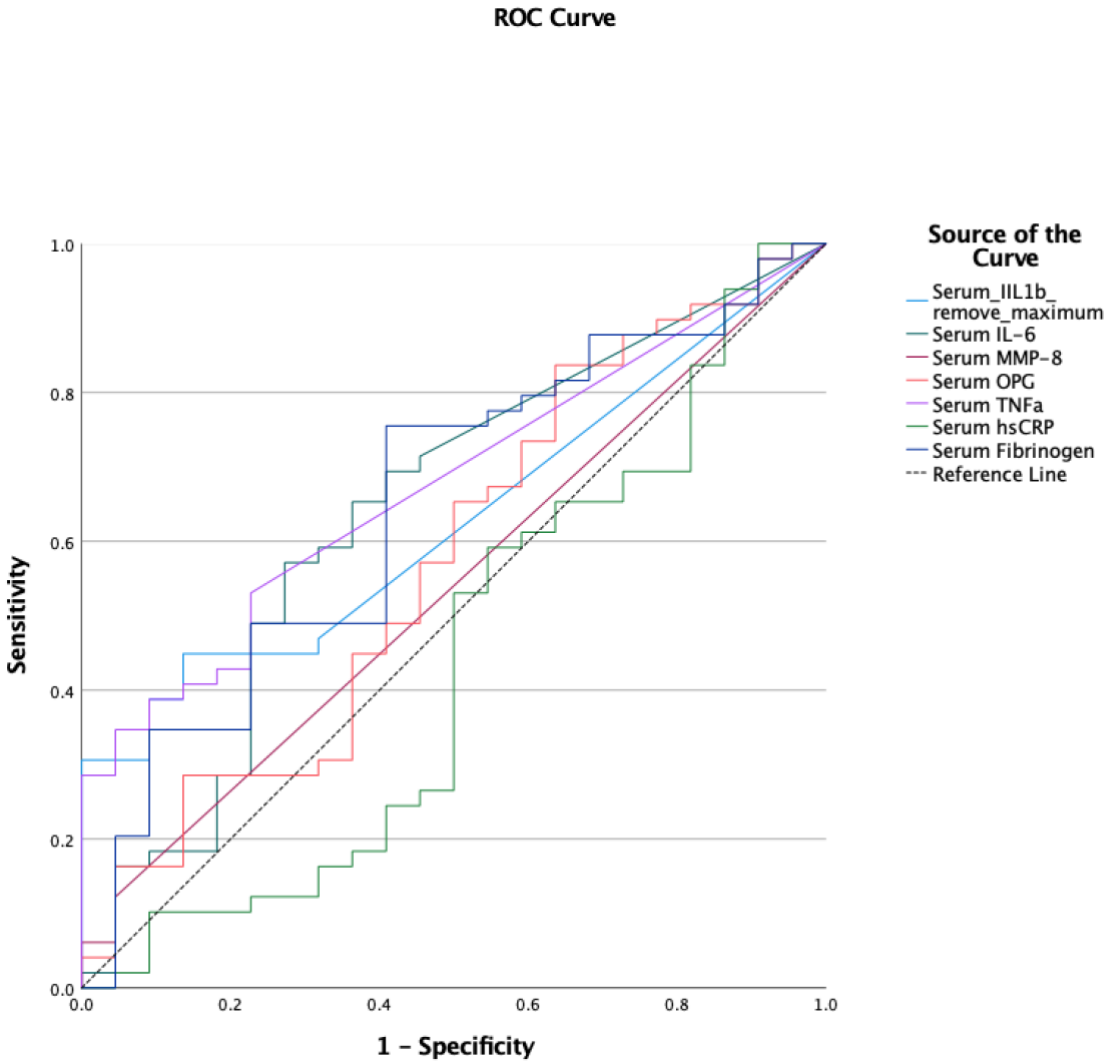


Table 13. Serum biomarkers among different comparison

	IL-1β (pg/ml)	**IL-6** (pg/ml)	**MMP-8** (pg/ml)	**OPG** (pg/ml)	**TNF-α** (pg/ml)	**hsCRP** (mg/L)	**Fibrinogen** (mg/dl)
non-CVD + Healthy implant	55.6±100.9	189.6±375.6	15.9±8.6	1142.1±989.1	32±67.3	8.2±4.4	55.6±23.1

non-CVD + mod/sev PI*	64.2±111.8	194.6±350.3	66.6±171.8	839.6±382.4	103.5±242.2	6.8±3.9	74.3±50.0
CVD + Healthy implant	142.2±184.4	352.2±655	42.8±76.8	1322.1±956.3	149.7±182.9	7±3.5	89.5±80.7
CVD + mod/sev PI*	60.7±62.9	191.6±479.8	16.8±11.2	1262±915.7	64.6±129.8	5.9±3.5	83.3±39.0
p-value	**0.03ǂ**	0.52	0.1	0.38	**0.03ǂ**	0.22	0.13
non-CVD + HI + Healthy perio	71.3±120.1	237.6±443.1	15.8±9.8	1184.2±1156.9	40±80.7	7.9±5.2	51.3±16.8
non-CVD + HI + Periodontitis	22.2±8	87.5±132	15.9±6.2	1057.8±590.3	15.1±12.1	8.6±2.7	63.6±31.8
non-CVD + mod-sev PI *+ Healthy perio	53.8±50.8	546.1±474.8	29.8±28.7	758.2±-	300.5±486.6	3.64±-	68.9±15.8
non-CVD + mod-sev PI* + Periodontitis	78.4±135.6	110.7±272.9	93.7±219.4	891.6±449.9	51.2±104.7	7.7±4	84.4±61.5
CVD + HI + Healthy perio	174±234.4	249.6±404.9	55.3±115.6	1720±1356	196.8±244.2	5.5±3.6	66.8±39.3
CVD + HI + Periodontitis	98.3±81.1	383.5±775.4	41.3±62.3	1219.8±711.7	94.8±97.5	7.3±3.4	85.9±53.9
CVD + mod -sev PI* + Healthy perio	51.5±58.2	127.4±194.7	13.2±0	721.1±218.5	85±108.5	3.7±4.2	88.7±49.9
CVD + mod-sev PI* + Periodontitis	58.3±60.2	192.6±509.4	16.9±11.8	1321.9±941.4	58.3±131.7	6.3±3.3	79.6±39
p-value	**0**.45	0.19	0.89	**0.04ǂ**	0.1	0.31	**001ǂ**
non-CVD+ Healthy implant	17.9±0	3.1±0	13.2±0	526.5±107.4	6.9±0	7.6±3.3	56.1±15.2
non-CVD+ PID	60.7±105.6	201.8±369.7	34.3±103.8	1080.2±864.3	59.2±155.9	7.7±4.4	62.7±36.7
CVD+ Healthy implant	224.9±270.6	488±673	82.6±134.5	1630.3±1025.6	209.1±204.2	8.5±2.4	13.6±14.7
CVD+ PID	84.0±126.4	240.8±559	23.1±34.7	1261.4±922.6	93.1±153.9	6.2±3.6	80.6±42.3
p-value	0.22	0.51	0.23	0.14	0.12	0.19	**0.01ǂ**
ASCVD low risk	50.2±88	104.7±253.5	24.3±20.7	976±1123.1	32.3±71.9	9.1±3.8	67.7±47.7
ASCVD borderline risk	81.2±82.4	150±207.6	15.5±6	1449.1±688.3	70.3±91.7	5.1±3.2	11.7±56.7
ASCVD intermediate risk	79.7±114.4	226±582.7	47.9±118.9	962.3±697.9	91.7±175.2	6.5±3.8	56.0±23.7
ASCVD high risk	94.4±144.8	292±530.3	24.9±50.1	1331.1±997.1	100.8±170	6.9±3.8	85.8±63.8
p-value	0.8	0.72	0.52	**0.04ǂ**	0.72	0.3	**0.02ǂ**

*mod-sev PI: moderate to severe peri-implantitis with bone loss≥ 2mm

ǂ denotes the significant difference between groups from Kruskal-Wallis test (P< 0.05)

Healthy perio= healthy periodontium

PID= peri-implant disease, including mucositis and peri-implantitis

ASCVD risk assessment: low-risk (<5%); borderline risk (5% to 7.4%); intermediate risk (7.5% to 19.9%); high risk (≥20%)

All serum biomarkers were measured in the unit of pg/ml, except hsCRP in mg/L and fibrinogen in mg/dl

Table 14. PICF biomarkers profiles between non-CVD and CVD group

	non-CVD		CVD		
Biomarker	mean(SD)	Median	mean (SD)	Median	p-value
CRP (pg/ml)	227.9±236.2	181.5	169.9± 171.1	130.7	0.27
IL-17 (pg/ml)	2.5±1.6	1.7	3.0 ±5.4	1.7	0.39
IL-1β (pg/ml)	129.8±94.0	118.6	101.8±110.7	55.0	**0.045**
IL-6 (pg/ml)	5.3±14.8	1.1	2.4±5.1	1.1	0.25
Il-8 (pg/ml)	283.8±143.4	306.1	275.9±147.6	293.4	0.77ǂ
MMP-8 (mg/L)	3144.9±985.1	3203.6	3333.2±1123.4	3476.8	0.17
MPO (pg/ml)	1135.6±608.8	1070.1	1284.1±781.1	1231.2	0.27
OPG (pg/ml)	82.4±127.7	35.2	78.1±200.5	15.5	0.11
TIMP-2 (pg/ml)	1365.9±698.2	1352.6	1278.8±741.3	1220.4	0.52ǂ
TNF-α(pg/ml)	8.3±3.2	6.6	11.3±8.0	6.6	**0.05**
VEGF (pg/ml)	101.0±116.7	66.0	74.7±101.0	43.2	0.11
PGE2 (pg/ml)	137.7±171.1	75.4	112.9±126.0	82.6	0.72

SD: standard deviation

Q1: 25th percentile, Q2: Median; Q3: 75th percentile

*Bold denotes the statistically significant difference (P<0.05) from Kruskal-Wallis test

ǂdenotes the statistically significant difference (P<0.05) from 1-way ANOVA test

Figure 2. ROC analysis of PICF TNF-α for CVD prediction

TNF-α	
AUC	0.59
95% CI	0.49-0.69
p-value	0.08
Optimal Cu-off value (Youden index) 6.8 pg/ml Sensitivity 46% Specificity 67%	

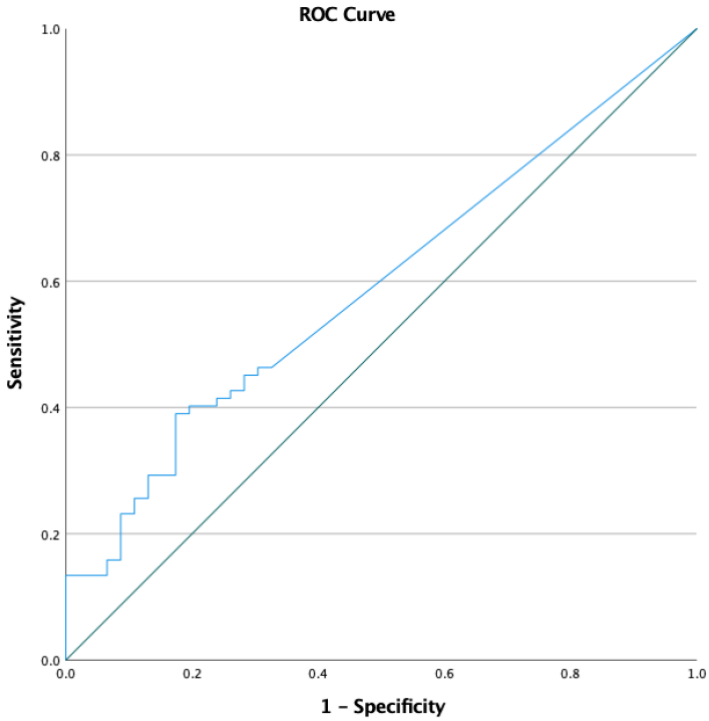


Table 15. PICF biomarkers profiles

	CRP	IL-17	IL-1β	IL-6	IL-8	MMP-8	MPO	OPG	TIMP-2	TNF-α	VEGF	PGE2
HI	192.6±172.9	2.6±2.1	115.9±97.3	2.7±5.3	275.3±138.9	3117.3±1044.1	1206.2±705.2	78±194.8	1275±682.5	9.8±5.7	85.4±103.7	120.1±138.3
Mod-sev PI	188.2±229.9	3.1±6.4	106.4±116.5	4.5±13.8	283.6±155.5	3468.7±1094.7	1264.3±756.6	81.8±151.6	1358.1±782.5	10.8±8.1	82.5±112.7	124.4±108.0
p-value	*0.46*	*0.21*	*0.35*	*0.39*	*0.79*	***0.05****	*0.75*	*0.44*	*0.63*	*0.85*	*0.23*	*0.39*
non-CVD+ HI	84.0±113.9	3.3±1.7	72.2±138.9	1.2±1.2	108.7±161.5	1695.1±1253.3	1418.6±1022.7	23.6±25.1	551.2±666.1	7.4±1.7	42.7±21.4	50.9±51.7
non-CVD+ PID	241.6±241.1	2.4±1.6	135.3±89.1	5.6±15.5	300.5±131.8	3282±851.7	1108.7±367.2	88.0±132.2	1443±657.0	8.3±3.3	106.5±120.6	146.4±176.7
CVD+ HI	139.7±171.3	2.6±1.4	52.5±81.0	1.1±0.0	202.2±157.2	2468.5±1368.5	943.6±739.2	42.0±56.0	757.7±684.9	9.8±3.7	55.3±76.1	94.9±63.5
CVD+ PID	173.2±172.0	3.0±5.7	107.1±112.5	2.5±5.4	283.9±145.4	3426.7±1063	1320.9±781.3	82.0±210.1	1335.1±729.2	11.4±8.3	76.8±103.5	115.0±94.4
p-value	0.27	0.11	**0.01***	0.5	0.07	**0.02***	0.41	0.25	**0.02***	0.27	0.26	0.85
HI	121.3±151.6	2.9±1.5	59.1±97.6	1.1±0.1	171.0±158.0	2210.7±1328.6	1101.9±829.2	35.9±47.4	688.8±655.7	8.9±3.3	51.1±62.0	80.3±61.4
PID	198.0±201.5	2.8±4.6	117.3±105.1	3.7±10.3	289.9±140.3	3374.7±990.4	1244.0±716.0	84.1±185.1	1374.3±703.0	10.3±7.1	87.6±11.4	126.4±130.5
p-value	0.2	**003***	**0.01***	0.44	**0.01**	**<0.01***	0.44	0.81	**<0.001***	0.89	0.22	0.28
HI	121.1±151.6	2.8±1.5	59±97.6	1.1±0.1	171±158	2210.7±1328.6	1101.9±829.2	35.9±47.4	688.8±655.7	8.9±3.3	51.1±62	80.3±61.4
MU	226.1±184.9	2.7±2.6	129.8±83.7	3.2±6.4	308±122.3	3452.3±813	1187.7±761.7	66.7±117.1	1397.8±526.3	10.4±6.4	91.9±83.8	133.6±121.4
PI	184.8±208.6	2.8 ±5.4	111.5±113.8	3.9±11.7	281.5±147.9	3338.3±1066.2	1270.4±697.1	92.3±209.7	1363.4±774.8	10.3±7.4	85.6±121.4	122.9±135.4
p-value	*0.2*	***0.04****	***0.01***	*0.38*	***0.03***	***0.01****	*0.57*	*0.96*	***<0.001****	*0.94*	*0.1*	*0.53*

HI: healthy implant; Mod-sev PI: moderate to severe per-implantitis (with bone loss ≥2mm); PID: peri-implant disease (including peri-implant mucositis and peri-implantitis); MU: peri-implant mucositis
*Bold denotes the statistically significant difference (P<0.05) from Kruskal-Wallis test

Table 16. GCF biomarkers profiles

		CRP	IL-17	IL-1 β	IL-6	IL-8	MMP-8	MPO	OPG	TIMP-2	TNF-α	VEGF
Periodontitis group	**Perio teeth**	293.5±219.6	1.3±0	160.2±108.9	4.2±17.7	134.8±39.3	3181.6.±711.7	1092.9±331.5	41.8±72.9	1322.2±532.5	13.7±9.8	97.5±112.3
	Heathy teeth	276±165.5	1.3±0.1	113.9±79.6	3.4±8.6	179.5±376.6	3109.1±664	1115±324.5	30.2±44.2	1311.4±484.7	16.1±13.4	73±58.8
Healthy group Healthy sites		302.8±214.6	1.4±0.2	146.1±112.3	3±5.8	141±41.5	3244.9±616.7	1128.5±372	47.8±86.7	1423.4±585.4	15.4±18.4	102.4±105.7
p-value		0.6	0.64	**0.01***	0.76	0.31	0.5	0.62	0.23	0.98	0.06	0.07

*Bold denotes the statistically significant difference (P<0.05) from Kruskal-Wallis test

Table 17. GCF comparison between CVD and control at different sites collecting GCF

		CRP	IL-17	IL-1β	IL-6	IL-8	MMP-8	MPO	OPG	TIMP-2	TNF-α	VEGF
Periodontitis teeth in P group	**Non-CVD**	319±177.7	1.3±0	154.6±94.1	9.2±30.9	136.3±34.4	3159.3±564.3	1091.7±295.4	41.5±90.4	1305.5±480.6	11.7±9.5	93.9±113.1
	CVD	282.8±228.8	1.3±0.1	154.5±112.2	1.7±1.5	134.8±39.3	3204.2±731.8	1098.2±329.3	40.1±60.5	1335.3±545.7	15.1±10.5	96.3±106.9
p-value		0.16	0.36	0.77	**0.02***	0.68	0.74	0.85	0.83	1	**0.02***	0.85
Healthy teeth in P group	**Non-CVD**	325±139.5	1.3±0	105±70.6	2.5±4.2	135.3±33.6	3121.3±678.2	1215.2±285.5	17.1±26.7	1215.8±490.1	11.6±7.9	68.7±52
	CVD	256.3±171.4	1.3±0.1	117.6±82.1	3.6±9.5	194.9±431.1	3114.4±630.3	1069.9±342.6	33.1±47.6	1361.7±459	18.7±14.9	71.1±60.4
p-value		0.06	0.35	0.68	0.56	0.85	0.93	0.28	0.23	0.2	**0.01***	1
Healthy teeth in H group	**Non-CVD**	391.6±226	1.3±0.0	176.4±122.8	4.1±10.1	146.9±44.9	3242±613.6	1148.3±386	51±71.4	1438.8±591.3	16±23.5	95.2±104.2
	CVD	247±170.1	1.3±0.1	130.8±91.2	2.2±2.3	130.9±33.9	3253.7±544.3	1170.9±361.7	32.5±59	1333.5±469.1	17.9±21	92±94.2
p-value		0.07	0.23	0.3	0.92	0.35	0.48	0.53	0.19	0.51	0.35	0.67
Healthy teeth in H group	**Non-CVD**	333.4±229.3	1.4±0.5	151.1±121.7	2.8±4.7	161.5±43	3405.4±651	1167±431.3	72±128.2	1549.2±596.2	12.5±13.1	104.2±85.3
	CVD	236.9±182.9	1.4±0.3	126.1±100.2	1.7±1	129±32.6	3064.7±625.5	1158.5±280.3	34.6±63.5	1257.9±657.4	14.7±16.9	98.8±146
p-value		0.12	0.18	0.48	0.79	**0.03***	0.3	0.84	0.27	0.27	0.5	0.19

*Bold denotes the statistically significant difference (P<0.05) from Kruskal-Wallis test

Figure 3. ROC analysis of GCF TNF-α for CVD prediction

TNF-α (periodontitis teeth in periodontitis patients)	
AUC	0.64
95% CI	0.51-0.78
p-value	0.04
Optimal Cu-off value (Youden index) 8.4 pg/ml Sensitivity 48% Specificity 83%	
TNF-α (healthy teeth in periodontitis patients)	
AUC	0.66
95% CI	0.54-79
p-value	0.05
Optimal Cu-off value (Youden index) 8.6 pg/ml Sensitivity 50% Specificity 83%	

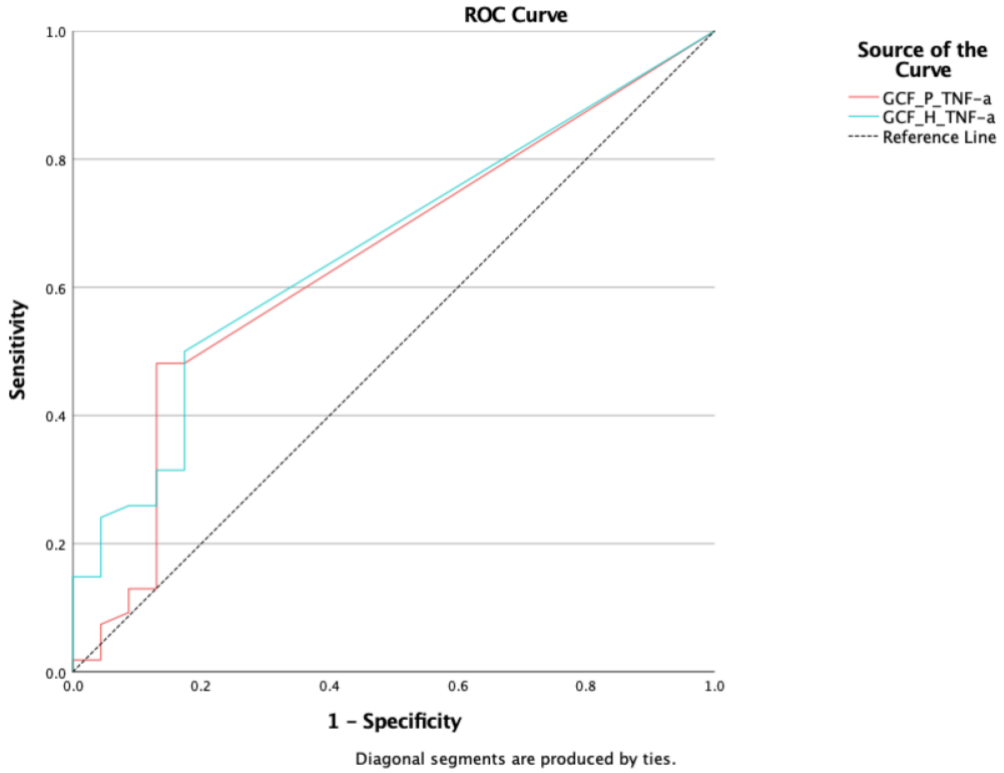


Figure 4. ROC analysis of GCF OPG for predicting moderate to severe peri-implantitis

OPG (periodontitis teeth in periodontitis patients)	
AUC	0.62
95% CI	0.50-75
p-value	0.06
Optimal Cu-off value (Youden index) 13.2 pg/ml Sensitivity 48% Specificity 72%	
OPG (healthy teeth in periodontitis patients)	
AUC	0.66
95% CI	0.54-79
p-value	0.05
Optimal Cu-off value (Youden index) 12.4 pg/ml Sensitivity 48% Specificity 73%	

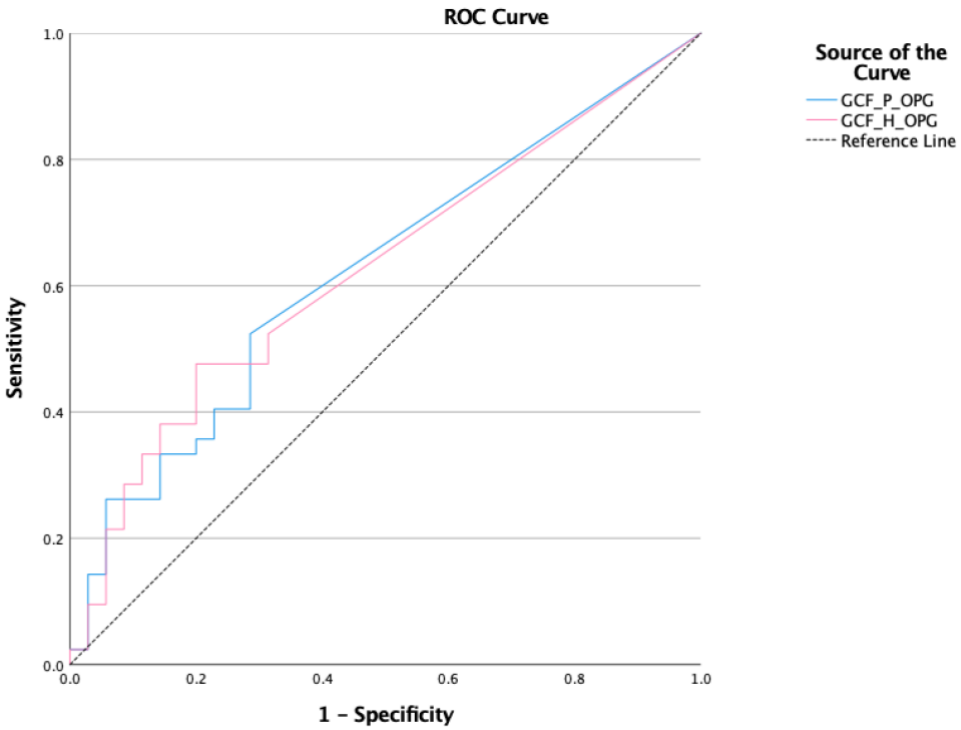


Table 18. Microbial compositions in the order of most commonly found between two groups

Total		non-CVD group		CVD group	
Taxonomy in species	**%**	**Taxonomy in species**	**%**	**Taxonomy in species**	**%**
*Fusobacterium nucleatum**	13.7	*Fusobacterium nucleatum**	16.1	*Fusobacterium nucleatum**	12.3
*Streptococcus mitis-oralis-sanguinis**	4.5	*Streptococcus mitis-oralis-sanguini*s*	4.0	*Streptococcus mitis-oralis-sanguinis**	4.7
Parvimonas micra	3.4	*Parvimonas micra*	3.7	*Streptococcus intermedius*	3.3
Streptococcus intermedius	3.4	*Streptococcus intermedius*	3.4	*Parvimonas micra*	3.3
Veillonella parvula	3.1	*Veillonella parvula*	3.0	*Veillonella parvula*	3.2
Prevotella oris	2.9	*Prevotella oris*	2.5	*Prevotella oris*	3.1
Unknown species	2.1	*Porphyromonas endodontalis*	2.3	*Haemophilus parainfluenzae*	2.7
Campylobacter gracilis	1.9	*Campylobacter gracilis*	2.0	*Unknow species*	2.3
Haemophilus parainfluenzae	1.9	*Clostridiales sp31630*	1.9	*Campylobacter gracilis*	1.9
Porphyromonas endodontalis	1.8	*Alloprevotella tannerae*	1.8	*Neisseria macacae-mucosa-sicca*	1.8
Neisseria macacae-mucosa-sicca	1.6	*Unknown species*	1.8	*Porphyromonas gingivalis*	1.7

* indicated the same most common two species in two groups

Table 19. Microbial compositions in the order of most commonly found in subgroups

non-CVD group				CVD group			
Healthy implant		Peri-implantitis		Healthy implant		Peri-implantitis	
Taxonomy in species	**%**	**Taxonomy in species**	**%**	**Taxonomy in species**	**%**	**Taxonomy in species**	**%**
*Fusobacterium nucleatum**	13.2	*Fusobacterium nucleatum**	17.0	*Fusobacterium nucleatum**	13.8	*Fusobacterium nucleatum**	11.5
Streptococcus mitis-oralis-sanguinis	5.5	*Parvimonas micra*	3.5	*Streptococcus mitis-oralis-sanguinis*	4.0	*Streptococcus mitis-oralis-sanguinis*	5.1
Streptococcus intermedius	4.2	*Streptococcus intermedius*	3.1	*Streptococcus intermedius*	3.6	*Veillonella parvula*	3.9
Parvimonas micra	3.8	*Streptococcus mitis-oralis-sanguinis*	3.1	*Unknown species*	3.3	*Prevotella oris*	3.6
Veillonella parvula	3.4	*Veillonella parvula*	2.9	*Neisserria macacae-mucosa-sicca*	3.0	*Parvimonas micra*	3.5
Porphyromonas endodontalis	2.6	*Prevotella oris*	2.5	*Parvimonas micra*	2.8	*Streptococcus intermedius*	3.2
Prevotella oris	2.6	*Unknown species*	2.3	*Haemophilus parainfluenzae*	2.3	*Haemophilus parainfluenzae*	2.9
Alloprevotella tannerae	2.4	*Oribacterium sp33063*	2.1	*Streptococcus sangiunis*	2.3	*Campylobacter gracilis*	1.9
Streptooccis mutans	2.3	*Clostridiales sp31630*	2.0	*Porphyromonas gingivalis*	2.2	*Unknown species*	1.8
Campylobacter gracilis	2.2	*Streptococcus anginosus*	1.9	*Prevotella oris*	2.1	*Porphyromonas endodontalis*	1.8

Table 20. Common Periodontal pathogens (%) in total amount of gene copies per sample

% in total amount	Non-CVD +HI	Non-CVD +PI	CVD +HI	CVD +PI
Fusobacterium nucleatum	13.2	17.0	13.8	11.5
Porphyromonas gingivalis	1.8	0.5	2.2	1.4
Tannerella forsythia	0.6	0.9	1.3	0.6
Prevotella intermedia	0.5	1.6	0.2	0.5
Treponema denticola	0.4	0.5	0.4	0.4

HI= healthy implant; PI= peri-implantitis (with detectable bone loss)

Table 21. Bacteria DNA counts *P. gingivalis, T. forsythia, and F. nucleatum obtained by RT-qPCR* between non-CVD and CVD group

	P. gingivalis	*T. forsythia*	*F. nucleatum*
	Mean±SD	Mean±SD	Mean±SD
Healthy	3.9 ± 3.4E+07	1.3 ± 1.5E+07	4.3 ± 9.4E+07
CVD	5.3 ± 6.5+07	1.5 ± 2.0+07	2.1 ± 3.0E+07
p-value	0.88	0.25	0.47
non-CVD + Healthy implant	2.3± 1.3E+07	0.8 ± 1E+07	4.1± 11.2E+07
non-CVD + Peri-implantitis(detectable bone loss)	4.5± 3.8E+07	1.6 ± 1.7E+07	4.6± 7.7E+07
CVD + Healthy implant	6.5± 8.8E+07	1.7±3.3E+07	1.6± 1.5E+07
CVD + Peri-implantitis (detectable bone loss)	4.8± 5.5E+07	1.4 ± 2.6E+07	2.6± 3.8E+07
p-value	0.97	0.26	0.38
non-CVD + HI + Healthy perio	1.6 ± 0.8E+07	0.8± 1.1E+07	5.8 ± 13.8E+07
non-CVD + HI + Periodontitis	3.7E+07	0.9 ± 0.7E+07	1 ± 1.2E+07
non-CVD + PI + Healthy perio	0.4E+07	2.2 ± 2.6E+07	3.2± 5.7E+07
non-CVD + PI + Periodontitis	5.2± 3.7E+07	1.3 ± 1E+07	6.2 ± 9.8E+07
CVD + HI + Healthy perio	2.1± 1.9E+07	0.8 ± 0.6E+07	1.1 ± 0.8E+07
CVD + HI + Periodontitis	12.1±10E+07	2.3 ± 4.2E+07	2.1 ± 1.8E+07
CVD + PI + Healthy perio	8.6± 3.8E+07	2.1 ± 4.1E+07	0.6 ± 0.6E+07
CVD + PI + Periodontitis	4.7± 5.8E+07	1.3 ± 2.3E+07	3.2 ± 4.3E+07
p-value	0.36	0.58	0.23

Table 22. Bacteria DNA counts of *P. gingivalis, T. forsythia, and F. nucleatum obtained by RT-qPCR of* different peri-implant disease

	P. gingivalis	*T. forsythia*	*F. nucleatum*

	Mean±SD	Mean±SD	Mean±SD
Healthy implant	5.8 ± 8E+07	1.3 ± 2.7E+07	2.8 ± 7.8E+07
Peri-implantitis with detectable bone loss	4.8 ± 5.2+07	1.5 ± 2.2+07	3.5 ± 5.9E+07
p-value	0.97	0.83	0.12
Healthy implant	5.2± 7.1E+07	1.3± 2.4E+07	3.2± 7.9E+07
Peri-implantitis with ≥ 2 mm bone loss	4.9± 5.3E+07	1.6 ± 2.5E+07	3.2± 3.6E+07
p-value	0.55	0.97	**0.04***
Healthy	5 ± 6.8E+07	1.2 ± 2.3E+07	2.9 ± 7.3E+07
Peri-implantitis with > 3 mm bone loss	5 ± 5.4E+07	2 ± 2.8E+07	4.1 ± 4.2E+07
p-value	0.51	0.29	**0.02***
Healthy	4.4 ± 5.9E+07	1.1 ± 2.1E+07	3.1 ± 7.2E+07
Peri-implantitis with > 4 mm bone loss	6.9 ± 6.4E+07	3.2 ± 3.3E+07	3.8 ± 2.7E+07
p-value	0.12	**0.01***	**0.03***
Healthy implant	1.6 ± 0.85E+07	0.33 ± 0.12E+07	0.64 ± 0.68E+07
Peri-implant mucositis	6.4 ± 8.5E+07	1.6 ± 3E+07	3.6 ± 9.2E+07
Peri-implantitis (detectable bone loss)	4.8 ± 5.2E+07	1.5 ± 2.3E+07	3.6 ± 5.9E+07
p-value	0.55	0.15	**0.02***
Healthy implant	1.6 ± 0.85E+07	0.33 ± 0.12E+07	0.64 ± 0.68E+07
Peri-implant disease	5.2 ± 6.2E+07	1.5 ± 2.5E+07	3.6 ± 7.3*E+07
p-value	0.8	0.28	**0.05***

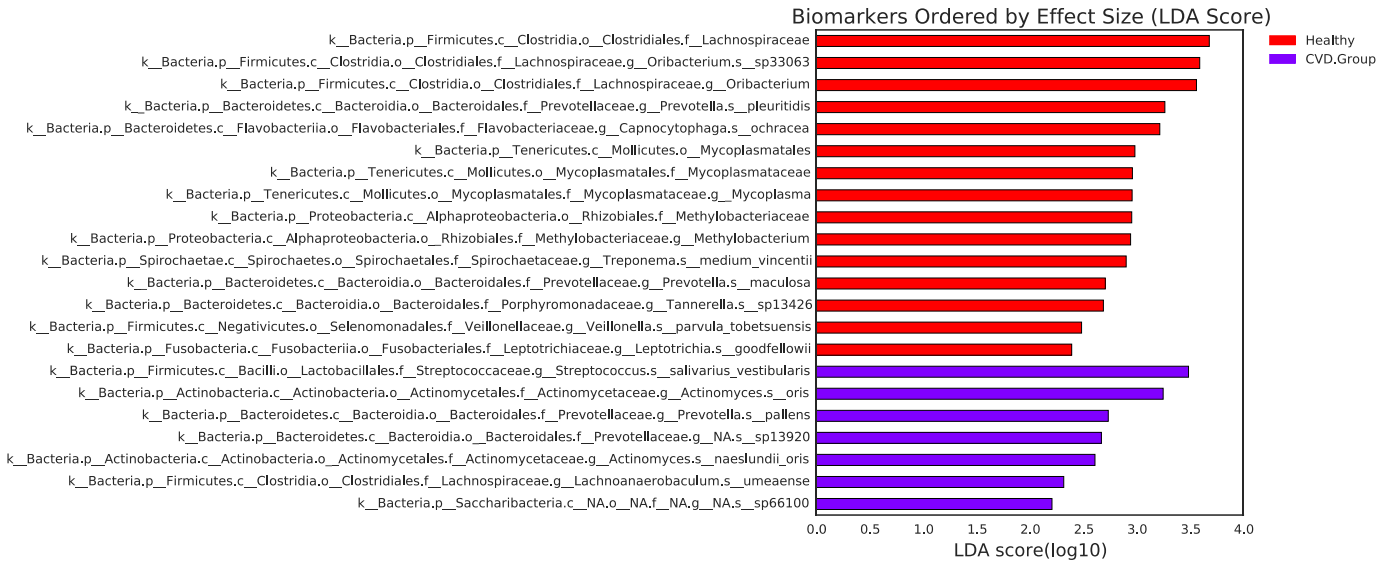
All analyses were dony by Kruskal-Wallis nonparametric test compared the median (Normality Shapiiro-Wilk test< 0.05)
*P-value in bold denotes the significance (p< 0.05)

Table 23. Patient characteristics of samples with detectable *Staphylococcus aureus*

	Age/ Gender	**Staph. aureus count in gene copies**	Case/Control	Peri-implant disease	Periodontal disease	Implant numbers in moutth	Deepest PPD	Suppuration	Function years	Maintenace	DM	Smoking
Patient A	66/F	**1.64E+04**	non-CVD	Incipient peri-implant bone loss	Healthy	Single	5 mm	Y	8	Regular	No	Never
Patient B	69/M	**1.64E+04**	CVD (Valve replacement> 8y)	Prei-implantitis with BL 4-6 mm (5 threads)	Full-moutth Stage 2	Multiple	7 mm	N	13	Episodic	Pre-diabetes	Ex- (>30y)
Patient C	73/F	**1.64E+04**	non-CVD	Prei-implantitis with BL 2-4mm (3 threads)	Local Stage 2	Multiple	10 mm	N	>15	Episodic	No	Ex- (>30y)
Patient D	75/M	**1.64E+04**	CVD (pacemaker> 6y)	Prei-implantitis with BL 4-6 mm (5 threads)	Full-moutth Stage 2	Single	6 mm	N	>5	Regular	Diabetes	Never

BL= bone loss

Figure 5. The prominent species among case and control groups with significant difference



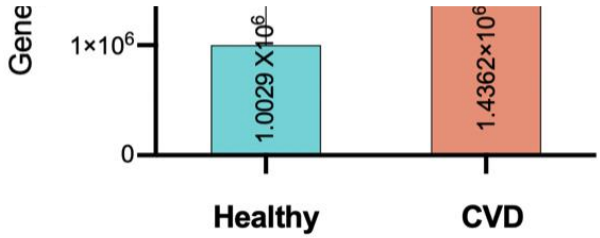


Figure 6. Absolute abundance among the "Case" and "Control" group

Figure 7. Alpha Diversity among CVD and non-CVD (Healthy) group

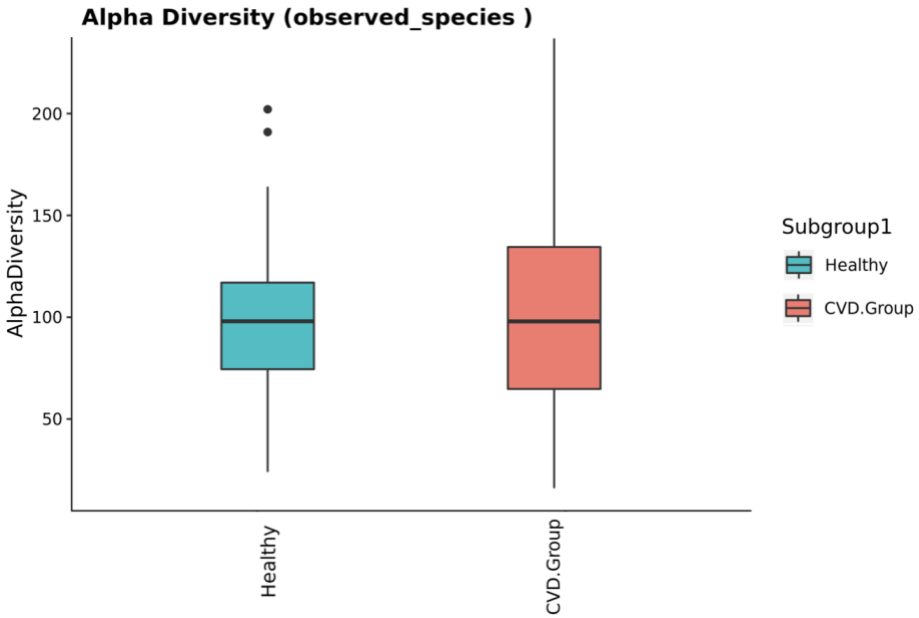
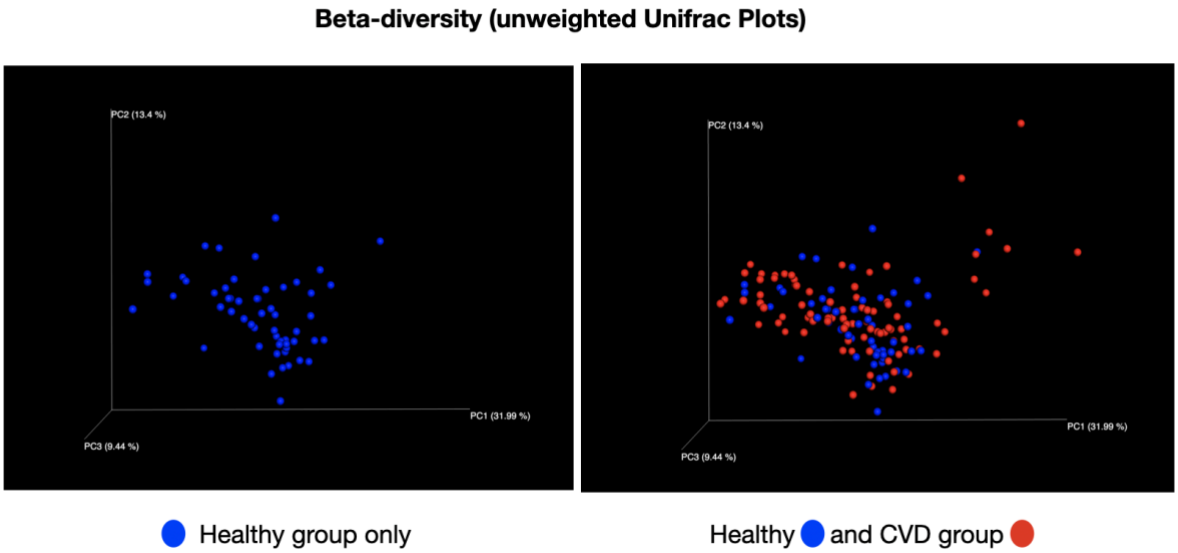


Figure 8. Beta-diversity among non-CVD (Healthy) and CVD group

Principal Coordinate Analysis of unweighted Unifrac distance of microbial communities associated to the non-CVD (Healthy)



and CVD group. Each dot represents an individual samples plots according to its microbial profile at genus level.

Figure 9. Mean value of absolute abundance in gene copies/µl among subgroups of healthy implants and peri-implantitis (with detectable bone loss) among the case and control groups

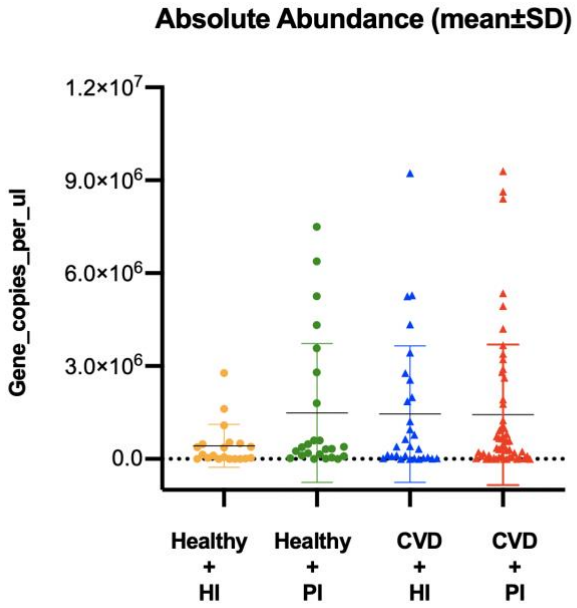
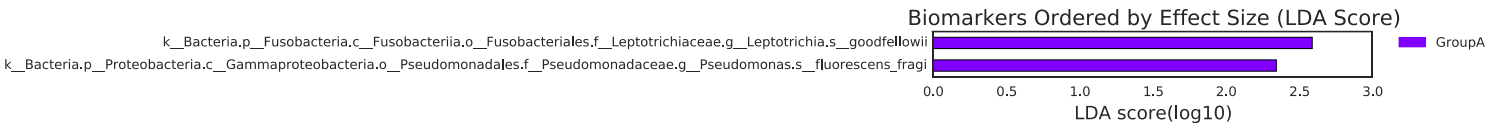


Figure 10. LEFse analysis among 4 subgroups between case and control groups case (with or without peri-implantitis)



*Group A= non-CVD + healthy implant
(No significant standout abundant species in the LEFse comparison: Group B= non-CVD + peri-implantitis; Group C= CVD + healthy
implant; Group D= CVD + per-implantitis)

Figure 11. LEFse analysis between case and control groups case (among subjects with peri-implantitis)

Group X= non-CVD+ Peri-implantitis Group Y= CVD+ Peri-implantitis

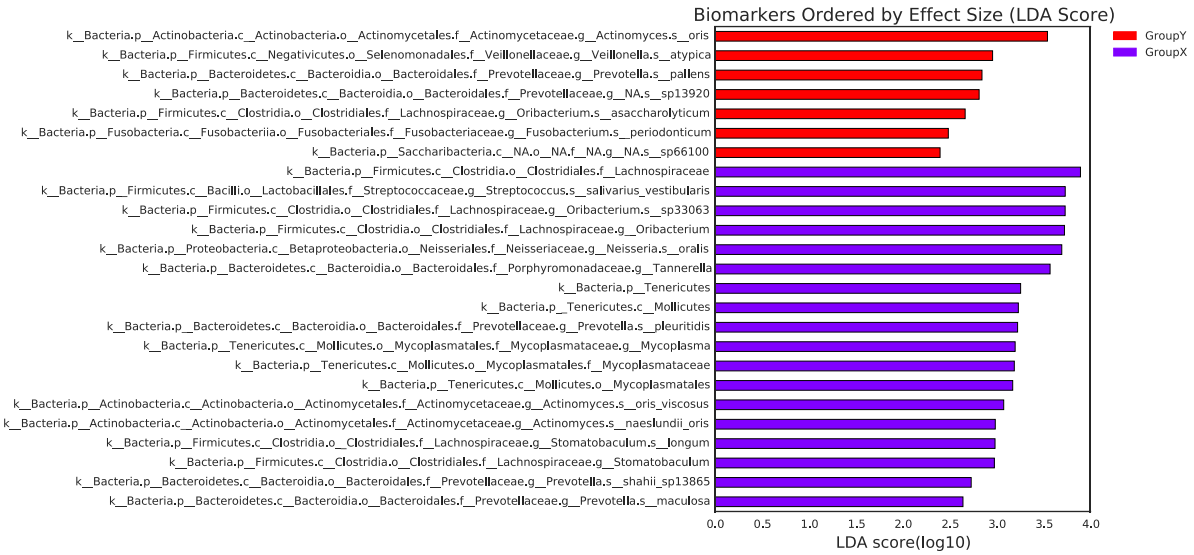


Figure 12. LEFse analysis between case and control groups case (among subjects with peri-implant disease)

Group A= non-CVD+ Peri-implant disease Group B= CVD+ Peri-implant disease

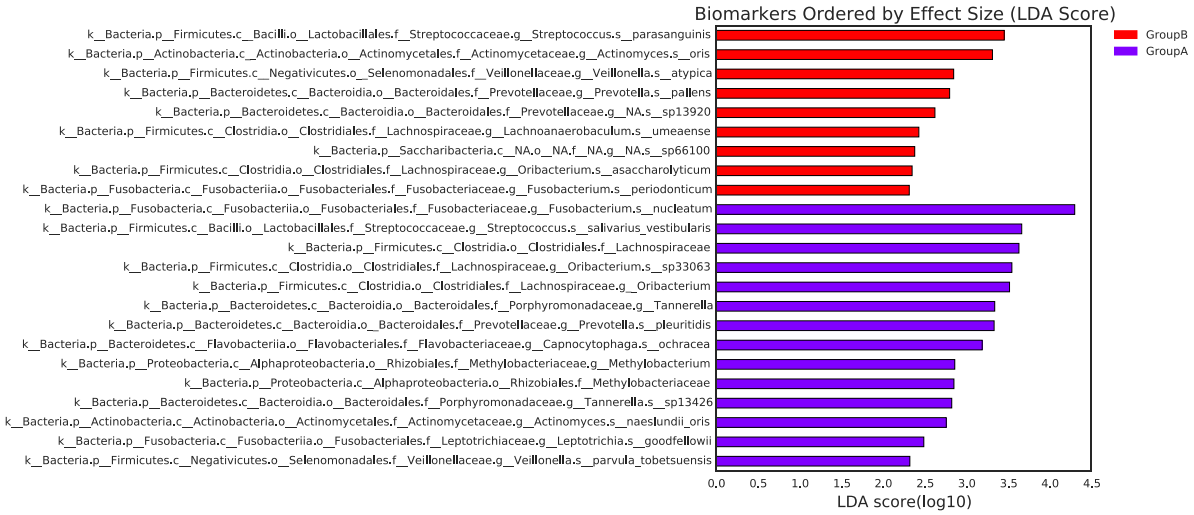


Figure 13. Beta-diversity among 4 subgroups (peri-implantitis between non-CVD and CVD group)

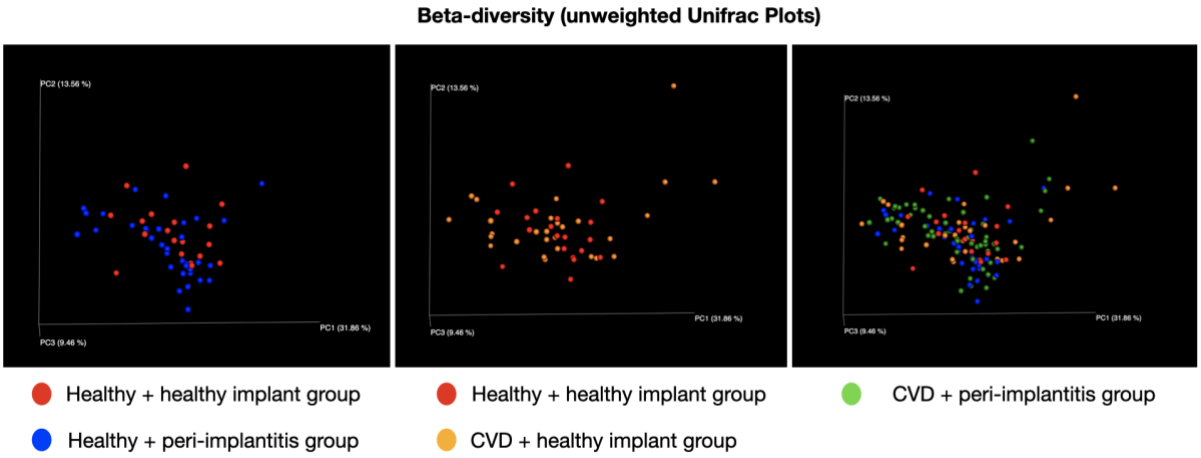


Figure 14. Beta-diversity comparison among non-CVD and CVD in peri-implantitis and peri-implant disease

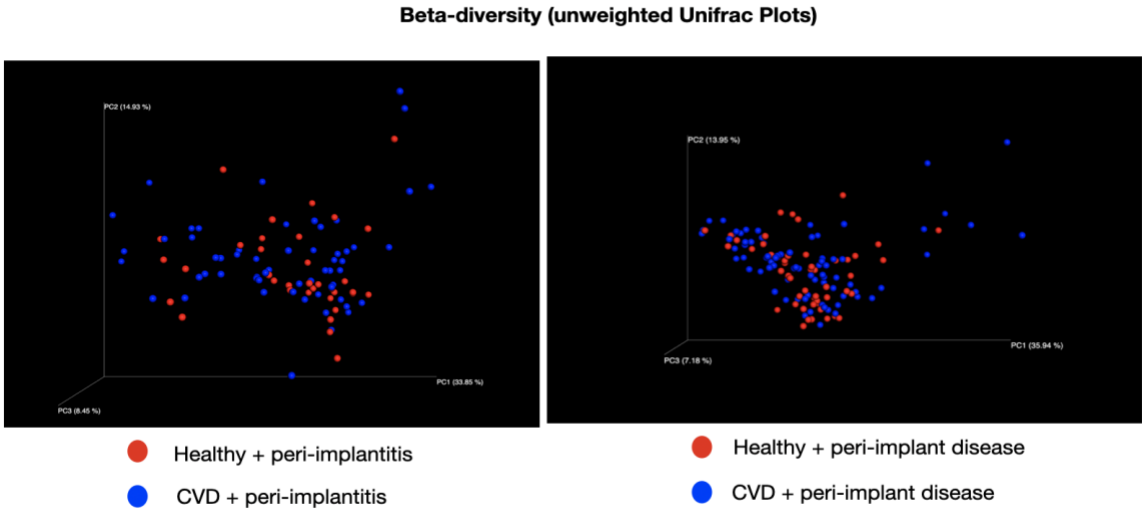
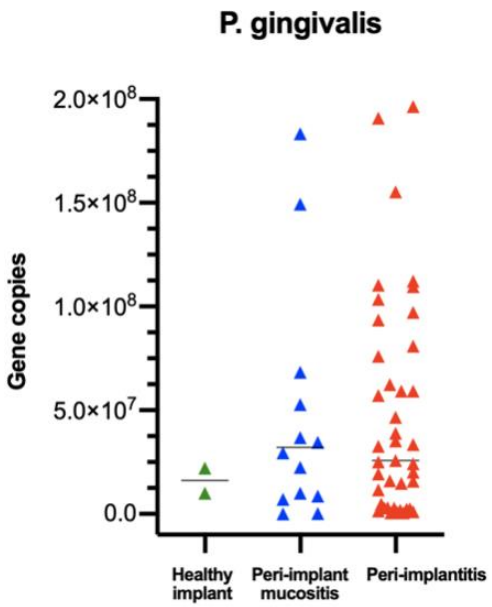
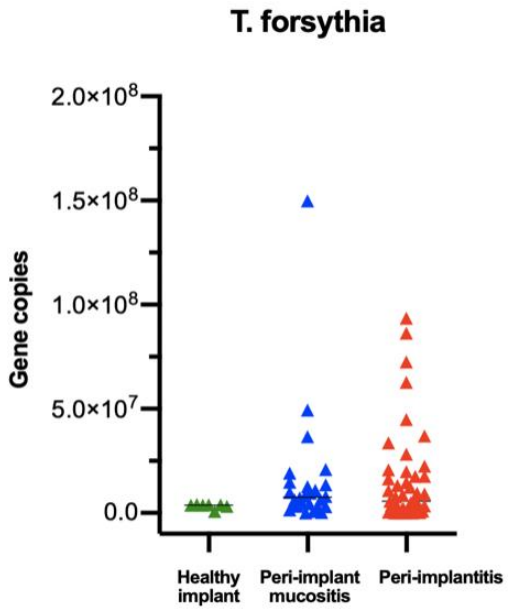
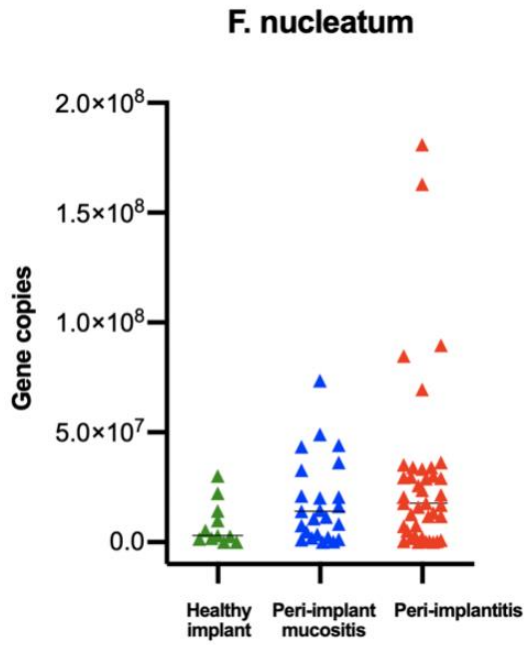


Figure 15. PCR results (bacterial counts) in the comparison of healthy and disease


P. gingivalis
Gene copies
2.0×10^8
1.5×10^8
1.0×10^8
5.0×10^7
0.0
Healthy implant
Peri-implant mucositis
Peri-implantitis


T. forsythia
Gene copies
2.0×10^8
1.5×10^8
1.0×10^8
5.0×10^7
0.0
Healthy implant
Peri-implant mucositis
Peri-implantitis


F. nucleatum
Gene copies
2.0×10^8
1.5×10^8
1.0×10^8
5.0×10^7
0.0
Healthy implant
Peri-implant mucositis
Peri-implantitis

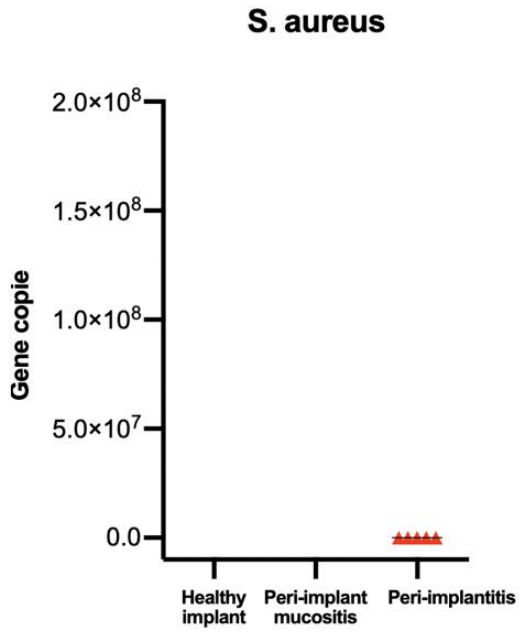
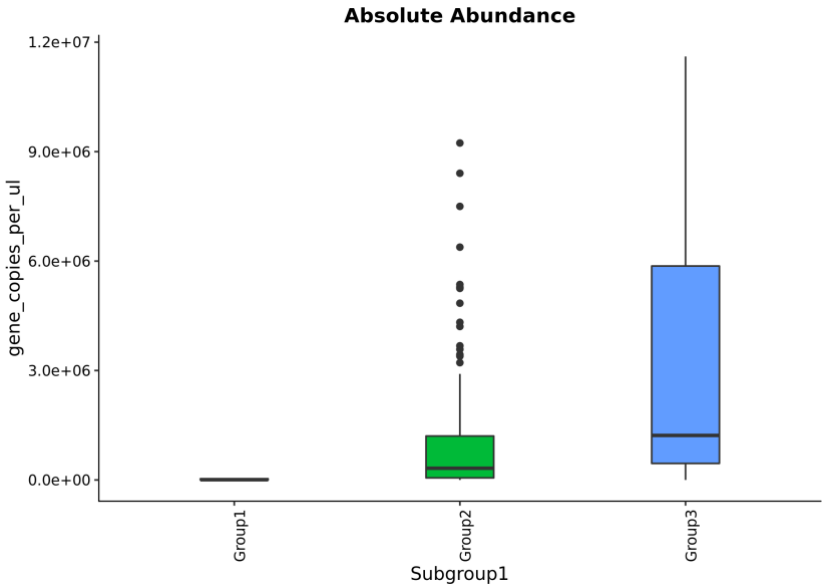

S. aureus
Gene copie
2.0×10^8
1.5×10^8
1.0×10^8
5.0×10^7
0.0
Healthy implant
Peri-implant mucositis
Peri-implantitis

Figure 16. Comparison of absolute abundance of different peri-implant pocket depths (PPD)



Group1: PPD≤ 3mm; Group2: PPD: 4-6 mm; Group 3: ≥ 7 mm

Figure 17. Microbial diversity among different peri-implant pocket depths in the cladogram and number of observed species

Group1: PPD≤ 3mm; Group2: PPD: 4-6 mm; Group 3: ≥ 7 mm

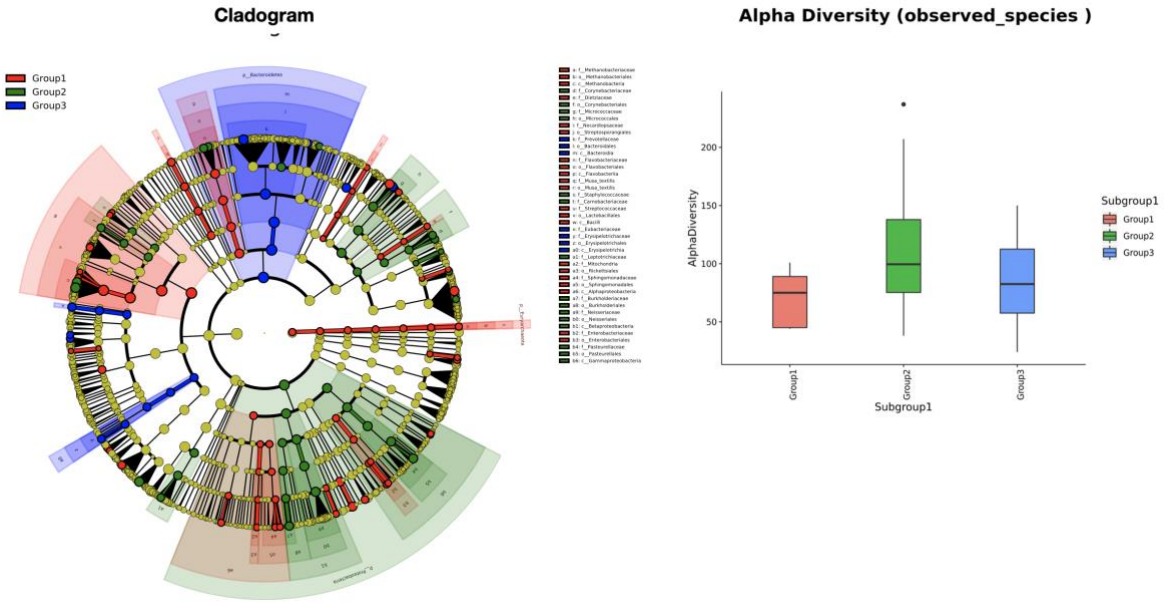
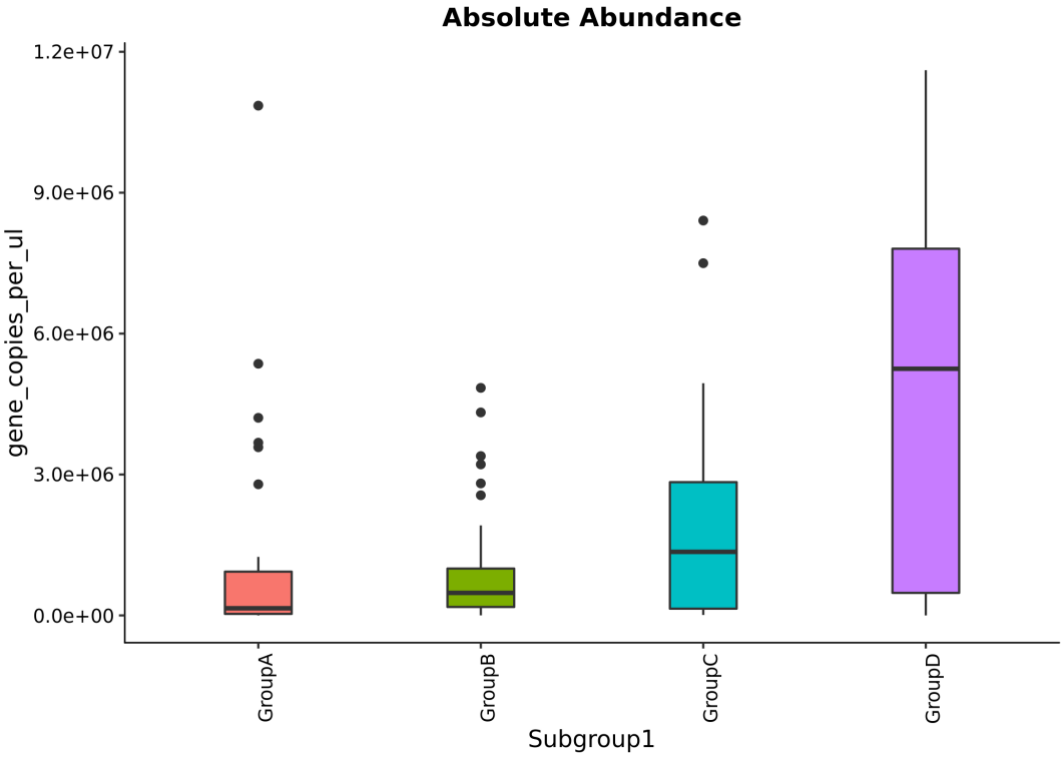
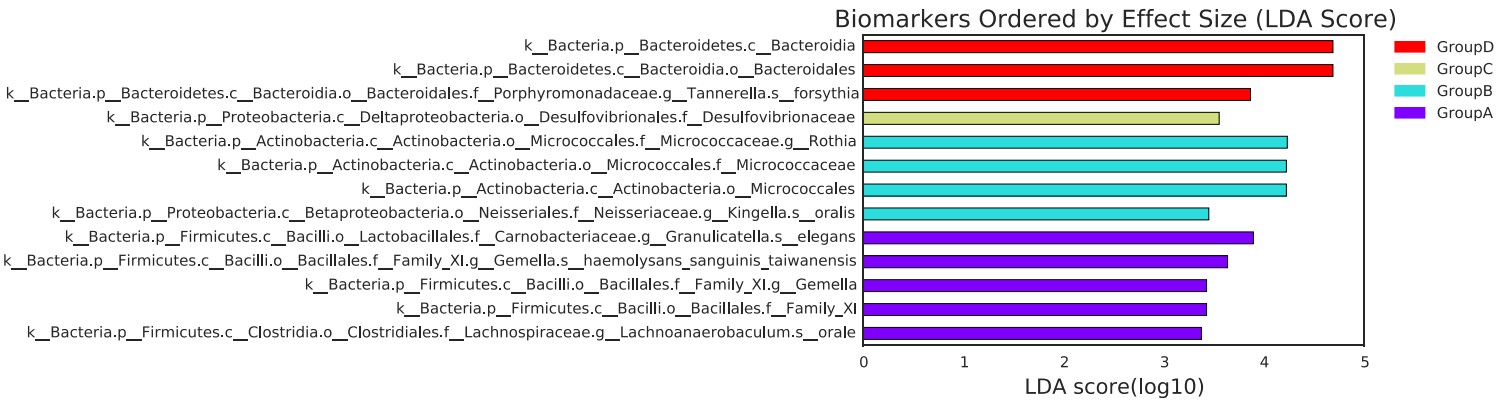


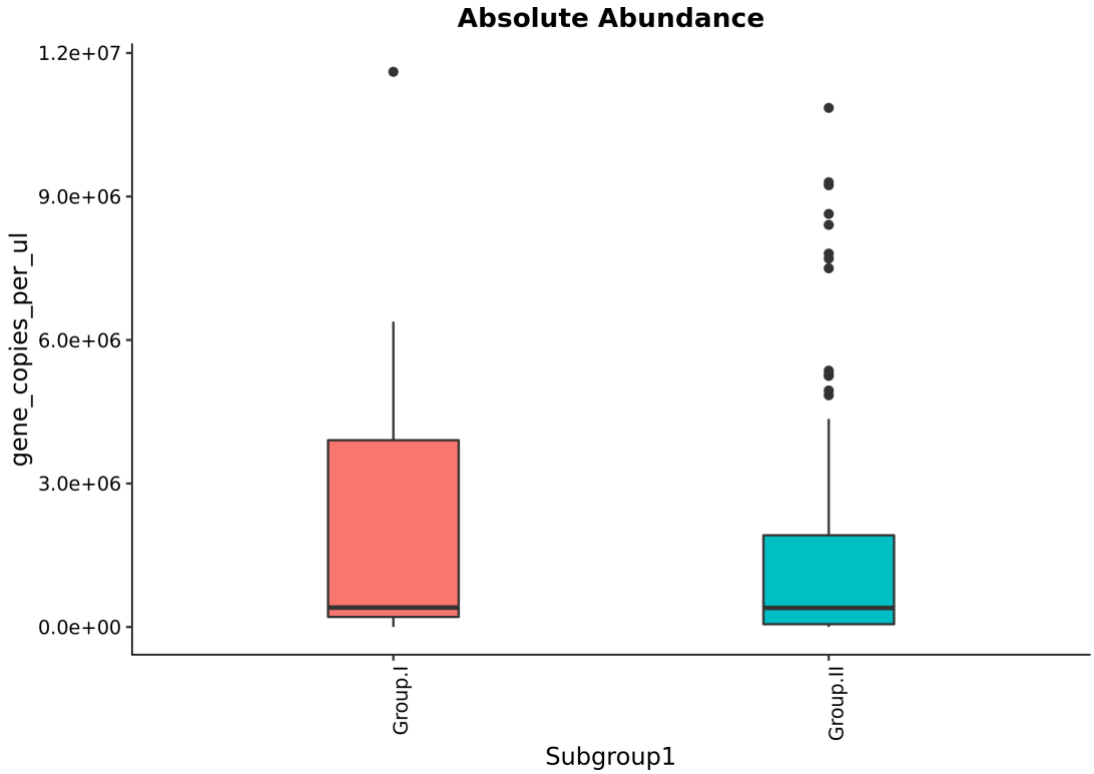
Figure 18. Absolute abundance among peri-implantitis with different bone loss level





Group A: bone loss <2mm; Group B: bone loss 2-4 mm; Group C: bone loss 4-6 mm; Group D: bone loss> 6 mm

Figure 19. Comparison of absolute abundance between presence and absence of suppuration

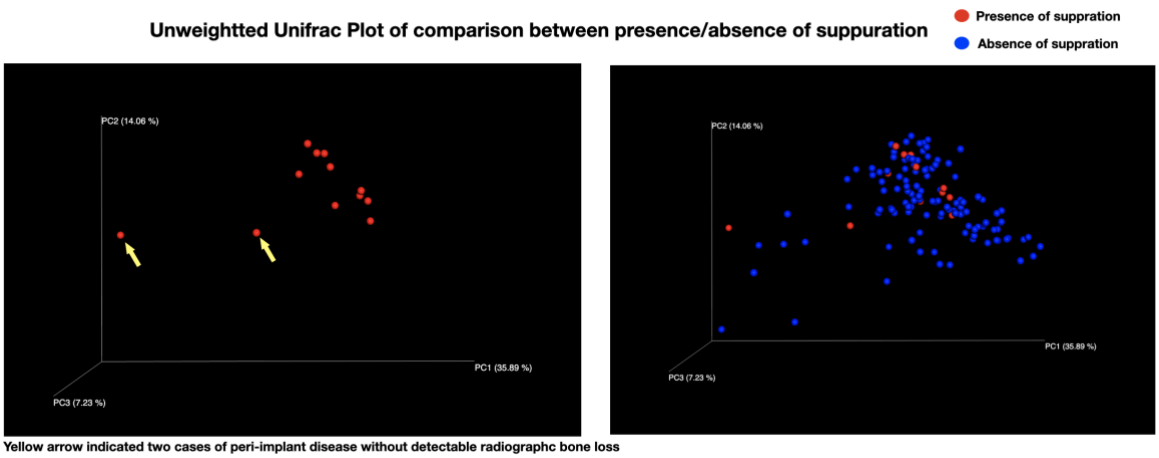


Group I: presence of suppuration; Group II: absence of suppuration

Figure 20. Beta Diversity metrics of microbial profile between presence/absence of suppuration

Figure 21. Microbial abundance and diversity between dentate and edentulous subjects

Group 1: dentate patients with healthy implant (*red*); Group 2: dentate patients with peri-implantitis (*green*)



Yellow arrow indicated two cases of peri-implant disease without detectable radiographc bone loss

Group 3: edentulous patients with healthy implant (*blue*); Group 4: edentulous patients with peri-implantitis (*purple*)

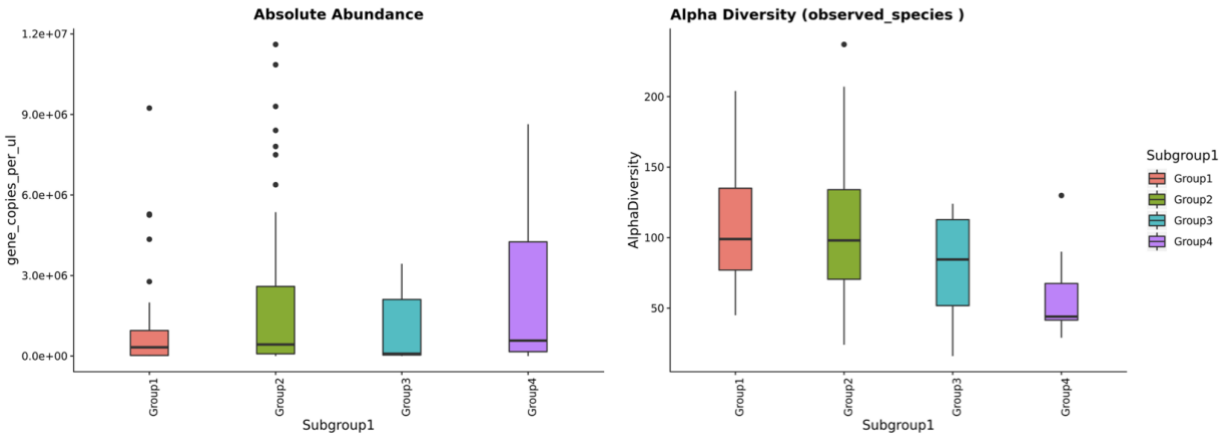


Figure 22. Cladogram of the distribution of microbial community in dentate/edentulous patients

Group 1: dentate patients with healthy implant (*red*); Group 2: dentate patients with peri-implantitis (*green*)
Group 3: edentulous patients with healthy implant (*blue*); Group 4: edentulous patients with peri-implantitis (*purple*)

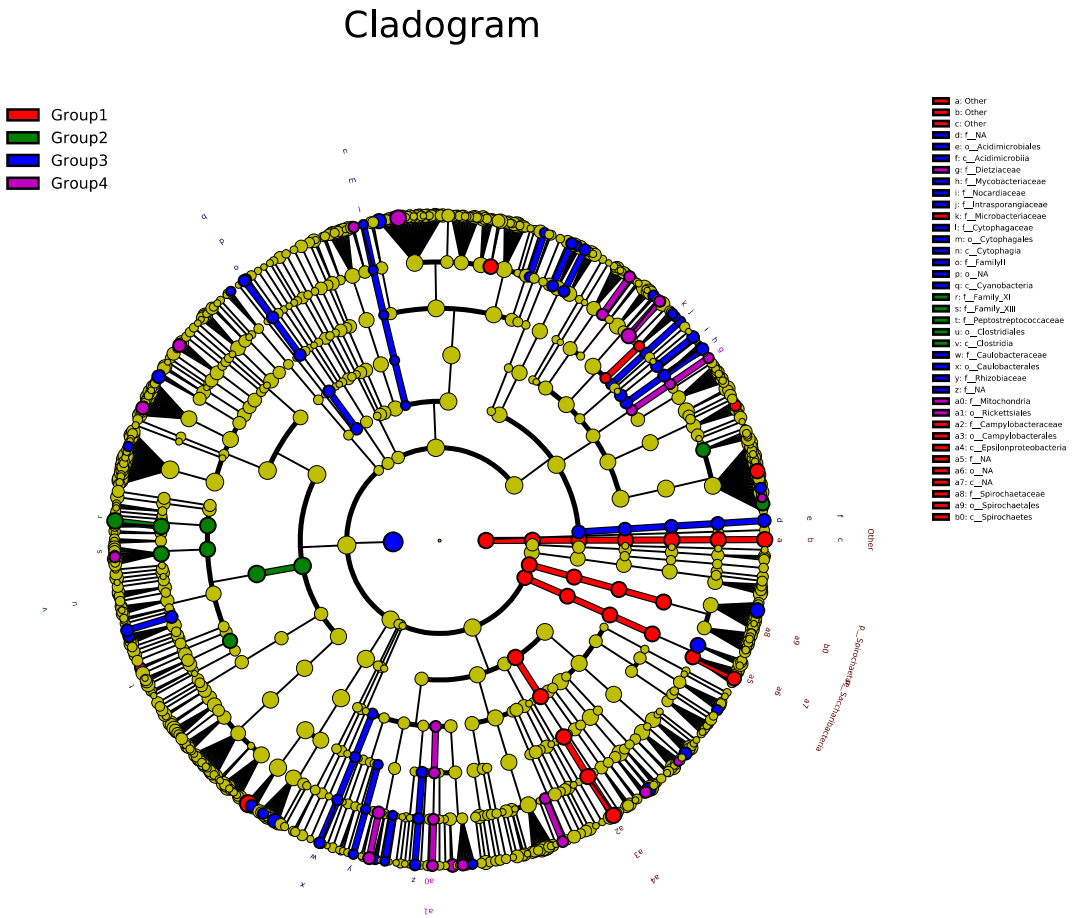


Figure 23. Simpson reciprocal index plot of alpha diversity (within sample) among peri-implantitis sites with simultaneous different periodontitis severity

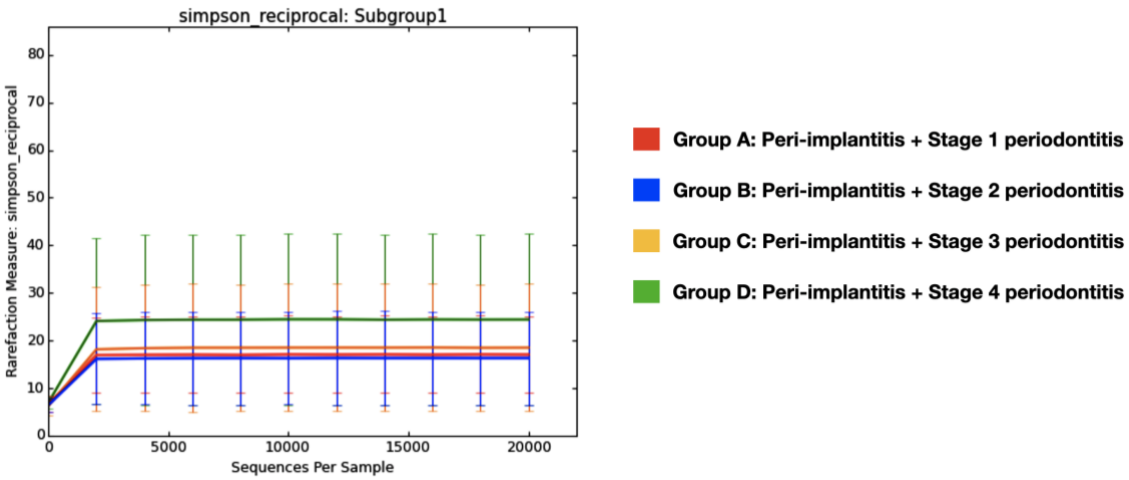


Figure 24. Microbial beta-diversity among pockets with peri-implantitis with simultaneous presence of different severity of periodontitis

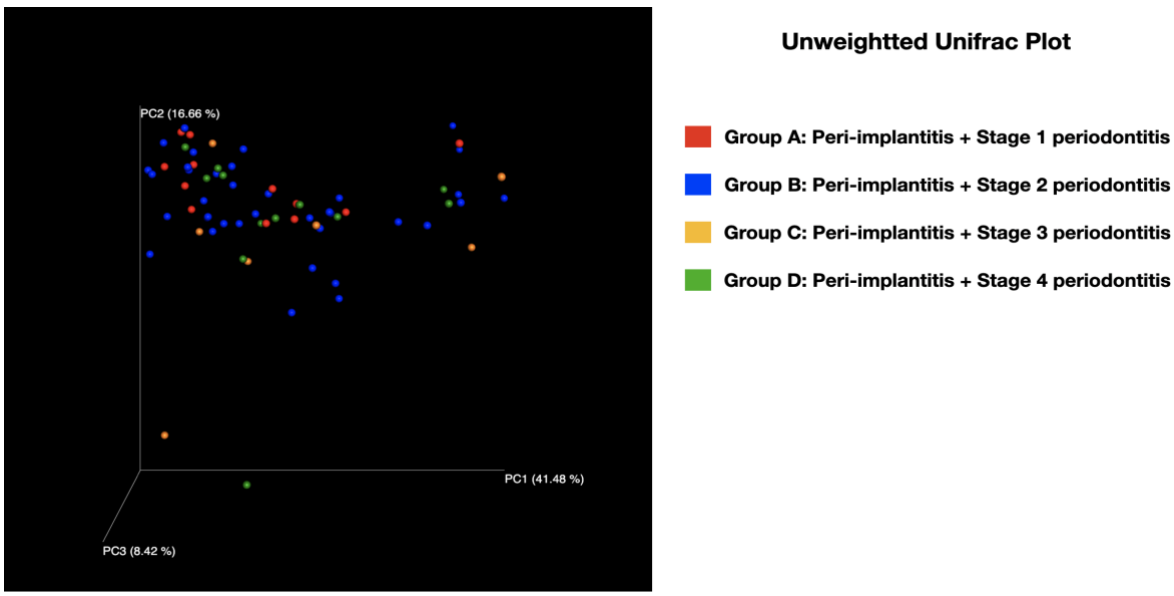


Table 24. Comparison of PICF biomarkers between moderate to severe peri-implantitis and healthy implants

*Bold denotes the statistically significant difference (P<0.05) from Kruskal-Wallis test

Biomarker	Healthy				Moderate to severe peri-implantitis				p-value
	Mean (SD)	Q1	Q2	Q3	Mean (SD)	Q1	Q2	Q3	
CRP	191.6±171.9	30	130.7	332.5	185.6±215.1	142.4	245.6	419.6	0.48
IL-17	2.5±2.1	1.7	1.7	2.7	3.1±5.9	1.7	1.8	4.3	0.3
IL-1β	117±99.5	21.1	102.8	179.2	99.5±111.9	53.8	168.1	248.2	0.19
IL-6	2.6±5.3	1.1	1.1	1.1	3.9±12.5	1.1	1.1	1.6	0.31
IL-8	273.9±140.6	174.4	293.4	393.2	281.2±155.6	304.9	398.8	468.8	0.76
MMP-8	3106.4±1050.7	2562.9	3302	3768.5	3387.2±1125.8	3476	4131.1	4636.8	0.09
MPO	1205.2±695.9	600.1	1111.5	1721.3	1267.1±769.4	1265.1	1854.1	2392.8	0.7
OPG	78.1±192.3	4.7	29.1	57.3	72.6±139.6	10.3	70.5	194.9	0.22
TIMP-2	1282.3±704.5	787.5	1243	1743.6	1267.7±777.4	1262.2	1799.5	2461.4	0.78
TNF-α	9.7±5.6	6.6	6.6	10.9	10.6±7.6	6.6	12.6	16.9	0.8
VEGF	87.5±104.9	32.6	55	93.9	73.6±105	35.3	90.9	165.6	**0.04***
PGE2	120.5±136.4	45.3	81.7	150.9	134.1±109.5	64.3	102.5	187.2	0.17

Table 25. Comparison of PICF biomarkers between peri-implant disease and healthy implants

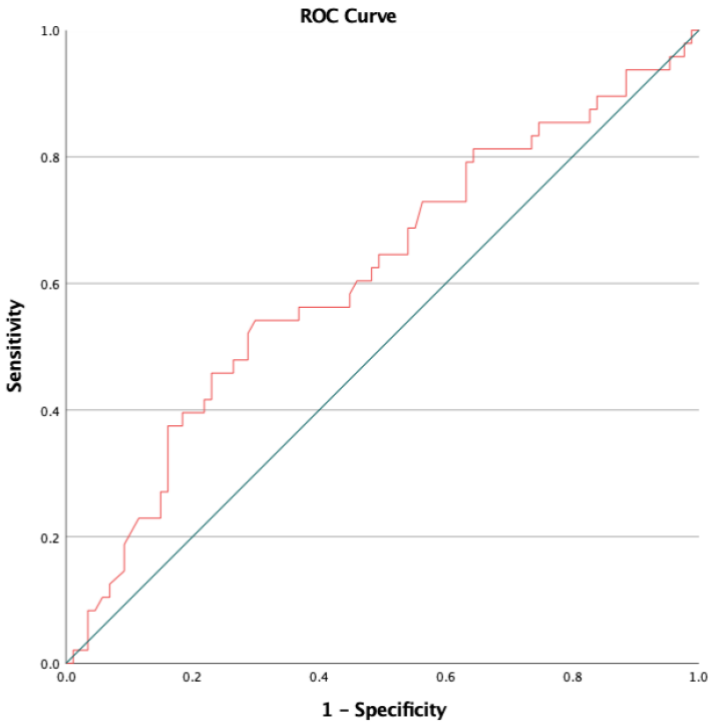
*Bold denotes the statistically significant difference (P<0.05) from Kruskal-Wallis test

Figure 25. ROC analysis of PGE-2 prediction ability for a more severe peri-implantitis (bone loss >3 mm)

Biomarker	Healthy				Periimplant disease				p-value
	Mean (SD)	Q1	Q2	Q3	Mean (SD)	Q1	Q2	Q3	
CRP	121.1±151.6	15.8	74.1	214.8	195±195.1	38.1	151.7	309.7	0.19
IL-17	2.8±1.5	1.7	2.2	3.7	2.8±4.5	1.7	1.7	1.9	0.3
IL-1β	59.1±97.6	3.5	8.1	76.4	113.5±105.3	21.6	75.1	178.8	**0.02***
IL-6	1.1±0.1	1.1	1.1	1.1	3.4±9.8	1.1	1.1	1.1	0.49
IL-8	171±158	17.3	114.4	301.615	287.1±142.9	176.9	304	395.2	**0.02***
MMP-8	2210.7±1328.6	1227.2	2176.6	3459.7	3331.6±1022	2721.4	3445.5	3952.5	**0.01***
MPO	1101.9±829.2	499.1	731.7	1664.8	1246.2±721.4	606.5	1199.5	1809.1	0.44
OPG	35.9±47.4	4.8	24.9	47.1	79.2±176	4.7	21.3	83	0.88
TIMP-2	688.8±655.7	161.2	435.3	1069.7	1329.7±722.1	777.8	1292.5	1799.5	**<0.01***
TNF-α	8.9±3.3	6.6	6.6	10.6	10.2±6.8	6.6	6.6	11.9	0.85
VEGF	51.1±62	10.6	40.1	61.9	83.8±107.6	23.2	50.6	95.6	0.30
PGE2	67.5±60.2	14.3	69.4	81.7	132.0±127.5	59.4	97.9	172.6	**0.04***

Figure 26. ROC analysis of MMP-8 prediction ability for severe peri-implantitis (bone loss >4mm)

PGE2	
AUC	0.61
95% CI	0.51-0.72
p-value	0.03
Optimal Cu-off value (Youden index) 91.6 pg/ml Sensitivity 58% Specificity 63%	



MMP-8	
AUC	0.62
95% CI	0.50-0.75
p-value	0.04
Optimal Cu-off value (Youden index) 3422.7 pg/ml Sensitivity 59% Specificity 63%	

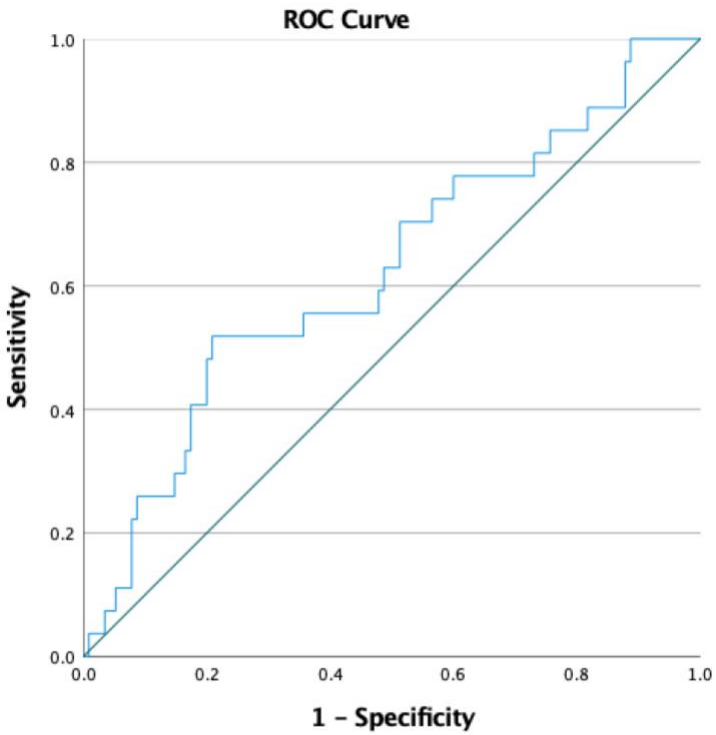
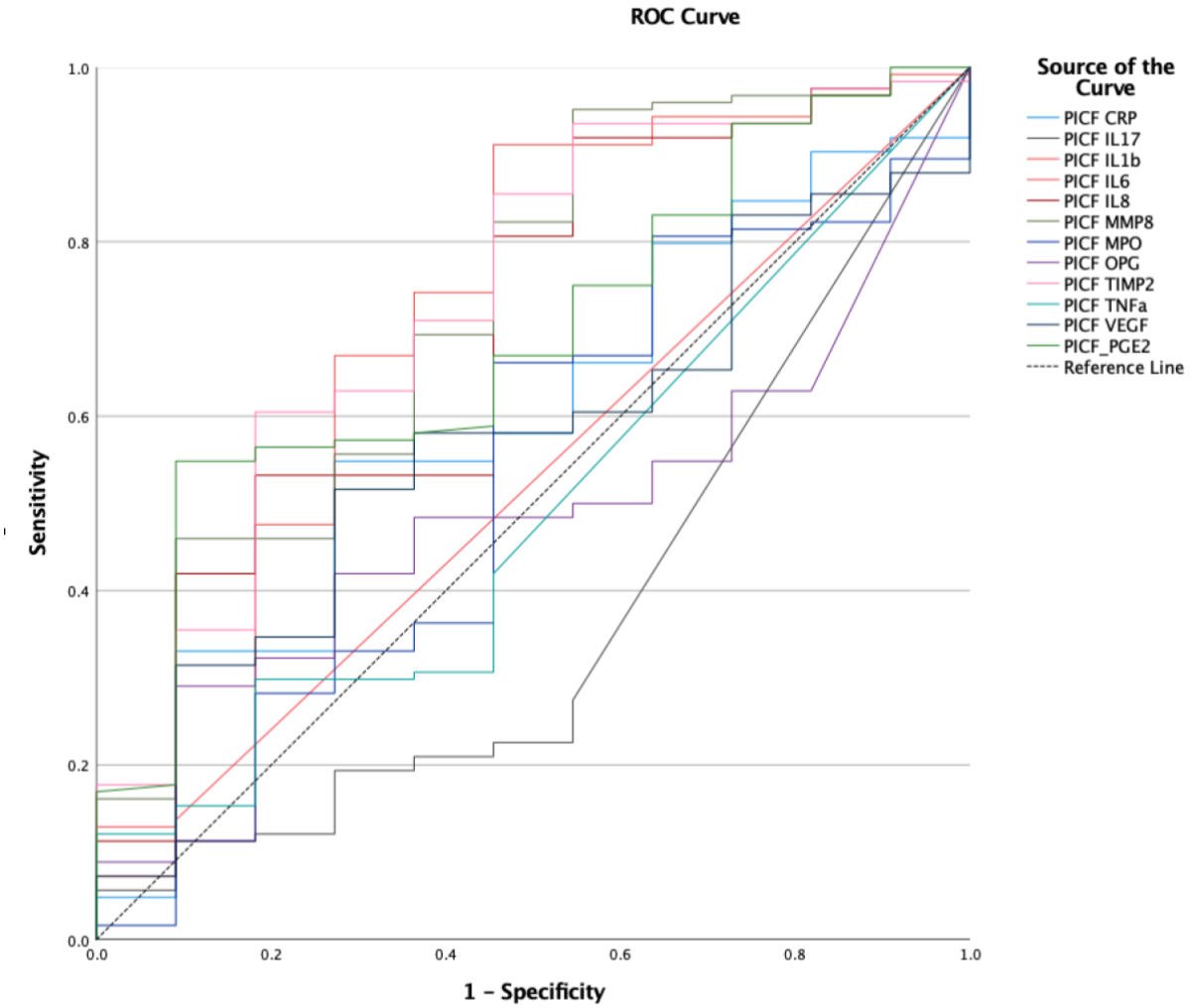


Figure 27. ROC analysis of PICF biomarkers for peri-implant disease



PICF Biomarker	AUC	95%	p-value
CRP	0.59	0.43-0.76	0.31
IL-17	0.37	0.20-0.55	0.16
IL1-b	0.70	0.52-0.90	**0.03**
IL6	0.54	0.38-0.70	0.75
IL8	0.70	0.53-0.89	**0.03**
MMP-8	0.73	0.56-0.90	**0.01**
MPO	0.53	0.34-0.71	0.79
OPG	0.49	0.40-0.63	0.92
TIMP-2	0.74	0.58-0.90	**0.01**
TNF-α	0.49	0.33-0.67	0.95
VEGF	0.57	0.42-0.73	0.41
PGE2	0.69	0.54-0.84	**0.04**

Table 26. PICF diagnostic ability for moderate to severe peri-implantitis and peri-implant disease

*Bold denotes the statistic significant difference (P<0.05) from χ^2 test
Mod-sev PI= moderate to severe peri-implantitis (bone loss≥ 2mm)
PID= peri-implant disease

Biomarker	Group	Threshold (pg/ml)	Marker level		Sensitivity	Specificity	OR	p-value
			High	Low				
MMP-8	Mod PI	3422.8	37	29	56.1%	55.3%	0.6	0.17
	Healthy implant		34	42				
IL-1β	Mod PI	71.6	26	40	39.4%	40.8%	2.2	**0.02***
	Healthy implant		45	32				
TIMP-2	Mod PI	1243.0	33	33	50%	50%	1	1.0
	Healthy implant		38	38				
PGE2	Mod PI	90.3	38	25	60.3%	53.6%	1.7	0.7
	Healthy implant		34	39				
MMP-8	PID		68	62	52.3%	75.0%	3.2	0.07
	Healthy implant	3422.8	3	9				
IL-1β	PID		68	62	52.30%	75.00%	3.3	0.07
	Healthy implant	71.6	3	9				
TIMP-2	PID		69	61	53.10%	83.30%	5.7	**0.03***
	Healthy implant	1243.0	2	10				
PGE2	PID		54	72	98.6%	15.6	13.1	**0.01***
	Healthy implant	90.3	1	10				

Figure 28. Predictability of peri-implant microbial profile

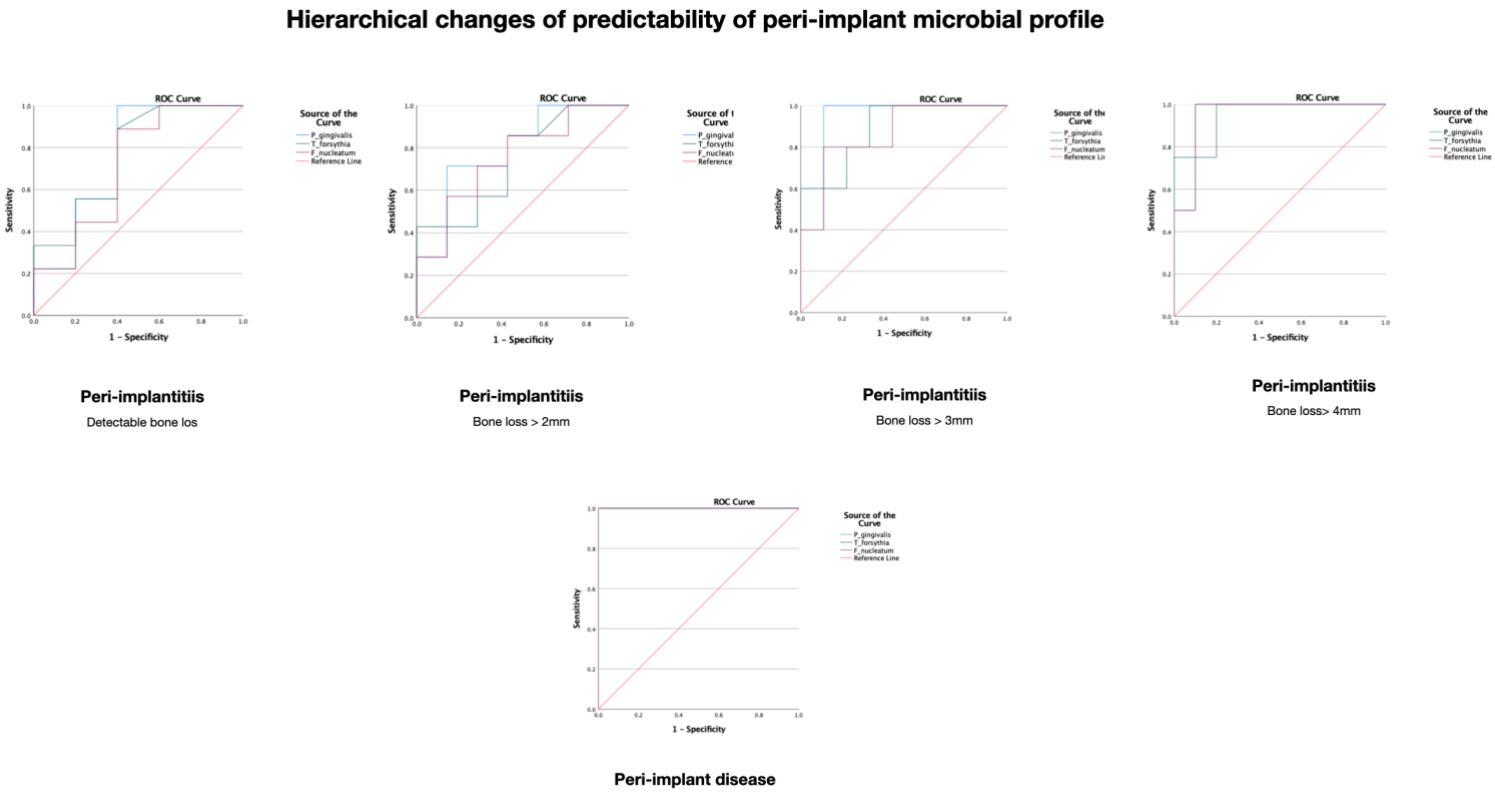


Table 27. ROC analysis of targeted species

	P. gingivalis		*T. forsythia*		*F. nucleatum*	
	AUC	p-value	AUC	p-value	AUC	p-value
Peri-implantitis (>detectable bone loss)	0.76	0.13	0.77	0.11	0.71	0.21
Moderate to severe peri-implantitis (≥2mm)	0.8	0.06	0.75	0.13	0.76	0.11
Severe peri-implantitis (>4mm)	0.95	**0.01**	0.95	**0.01**	0.95	**0.01**
peri-implant disease	1.0	**0.03**	1.0	**0.03**	1.0	**0.03**

Table 28. Diagnostic ability of targeted bacterial species

*Bold denotes the statistic significant difference (P<0.05) from χ^2 test; Mod-sev PI= moderate-severe peri-implantitis (bone loss≥2 mm); OR= odds ratio

Biomarker	Group	Sensitivity	Specificity	OR	p-value
P. gingivalis	Mod-sev PI Healthy implant	56.1%	55.6%	1.6	0.35
T. forsythia	Mod-sev PI Healthy implant	63.9%	54.7%	2.1	0.08
F. nucleatum	Mod-sev PI Healthy implant	69%	57.7%	3.0	**0.02***
P. gingivalis	Peri-implant disease Healthy implant	53.8	100%	8.1	0.17
T. forsythia	Peri-implant disease Healthy implant	56.6%	100%	16.9	**0.01***
F. nucleatum	Peri-implant disease Healthy implant	57.1%	81.8%	6.0	**0.02***

Table 29. Combined diagnostic ability of selected PICF biomarkers and targeted bacterial species

Biomarker	Group	Sensitivity	Specificity	OR	p-value
P. gingivalis + MMP-8	Mod-sev peri-implantitis Healthy implant	59%	50%	1.5	0.54
P. gingivalis + IL-1β	Mod-sev peri-implantitis Healthy implant	43%	55%	1.0	0.96
P. gingivalis + TMP-2	Mod-sev peri-implantitis Healthy implant	53%	53%	1.3	0.65
T. forsythia + MMP-8	Mod-sev peri-implantitis Healthy implant	71%	53%	2.9	0.08
T. forsythia + IL-1β	Mod-sev peri-implantitis Healthy implant	65%	47%	1.7	0.38
T. forsythia + TMP-2	Mod-sev peri-implantitis Healthy implant	65%	53%	2.1	0.23
F. nucleatum + MMP-8	Mod-sev peri-implantitis Healthy implant	77%	63%	5.6	**0.01***
F. nucleatum + IL-1β	Mod-sev peri-implantitis Healthy implant	82%	52%	5.0	**0.02***
F. nucleatum + TMP-2	Mod-sev peri-implantitis Healthy implant	77%	55%	4.0	**0.03***
P. gingivalis + MMP-8	Peri-implant disease Healthy implant	43%	55%	6.89	0.11
P. gingivalis + IL-1β	Peri-implant disease Healthy implant	47.4%	100%	6.3	0.11
P. gingivalis + TMP-2	Peri-implant disease Healthy implant	53.8%	100%	5.8	0.14
T. forsythia + MMP-8	Peri-implant disease Healthy implant	63%	100%	18.5	**0.05***
T. forsythia + IL-1β	Peri-implant disease Healthy implant	63.3%	100%	18.7	**0.01***
T. forsythia + TMP-2	Peri-implant disease Healthy implant	57.4%	100%	12.1	**0.03***
F. nucleatum + MMP-8	Peri-implant disease Healthy implant	58.5%	100%	18.2	**0.01***
F. nucleatum + IL-1β	Peri-implant disease Healthy implant	66.7%	100%	21.6	**<0.01***
F. nucleatum + TMP-2	Peri-implant disease Healthy implant	61.9%	100%	14.5	**0.02***

*Bold denotes the statistic significant difference ($P<0.05$) from χ^2 test

OR= odds ratio

Mod-sev peri-implantitis= moderate to severe peri-implantitis (bone loss≥2 mm)

Chapter VIII. References

Aarabi G, Thomalla G, Heydecke G, Seedorf U. 2019. Chronic oral infection: An emerging risk factor of cerebral small vessel disease. Oral Diseases. 25(3):710-719.

Agca R, Heslinga SC, Rollefstad S, Heslinga M, McInnes IB, Peters MJ, Kvien TK, Dougados M, Radner H, Atzeni F et al. 2017. Eular recommendations for cardiovascular disease risk management in patients with rheumatoid arthritis and other forms of inflammatory joint disorders: 2015/2016 update. Ann Rheum Dis. 76(1):17-28.

Ahn Y-B, Shin M-S, Han D-H, Sukhbaatar M, Kim M-S, Shin H-S, Kim H-D. 2016. Periodontitis is associated with the risk of subclinical atherosclerosis and peripheral arterial disease in korean adults. Atherosclerosis. 251:311-318.

Ait-Oufella H, Taleb S, Mallat Z, Tedgui A. 2011. Recent advances on the role of cytokines in atherosclerosis. Arterioscler Thromb Vasc Biol. 31(5):969-979.

Al-Majid A, Alassiri S, Rathnayake N, Tervahartiala T, Gieselmann DR, Sorsa T. 2018. Matrix metalloproteinase-8 as an inflammatory and prevention biomarker in periodontal and peri-implant diseases. Int J Dent. 2018:7891323.

Alberti K, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart J-C, James WPT, Loria CM, Smith Jr SC. 2009. Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; american heart association; world heart federation; international atherosclerosis society; and international association for the study of obesity. Circulation. 120(16):1640-1645.

Albertini M, López-Cerero L, O'Sullivan MG, Chereguini CF, Ballesta S, Ríos V, Herrero-Climent M, Bullón P. 2015. Assessment of periodontal and opportunistic flora in patients with peri-implantitis. Clin Oral Implants Res. 26(8):937-941.

American Diabetes Association. 2010. Standards of medical care in diabetes--2010. Diabetes Care. 33 Suppl 1(Suppl 1):S11-61.

My life check-life's simple 7 [accessed]. https://www.heart.org/en/professional/workplace-health/lifes-simple-7.

Anderson DR, Poterucha JT, Mikuls TR, Duryee MJ, Garvin RP, Klassen LW, Shurmur SW, Thiele GM. 2013. Il-6 and its receptors in coronary artery disease and acute myocardial infarction. Cytokine. 62(3):395-400.

Aoyama N, Suzuki JI, Kobayashi N, Hanatani T, Ashigaki N, Yoshida A, Shiheido Y, Sato H, Minabe M, Izumi Y. 2018. Associations among tooth loss, systemic inflammation and antibody titers to periodontal pathogens in japanese patients with cardiovascular disease. Journal of periodontal research. 53(1):117-122.

Araujo MG, Lindhe J. 2018. Peri-implant health. Journal of periodontology. 89(S1):S249-S256.

Armingohar Z, Jørgensen JJ, Kristoffersen AK, Abesha-Belay E, Olsen I. 2014. Bacteria and bacterial DNA in atherosclerotic plaque and aneurysmal wall biopsies from patients with and without periodontitis. J Oral Microbiol. 6.

Ata-Ali J, Flichy-Fernández AJ, Alegre-Domingo T, Ata-Ali F, Palacio J, Peñarrocha-Diago M. 2015. Clinical, microbiological, and immunological aspects of healthy versus peri-implantitis tissue in full arch reconstruction patients: A prospective cross-sectional study. BMC Oral Health. 15:43.

Augthun M, Conrads G. 1997. Microbial findings of deep peri-implant bone defects. The International journal of oral & maxillofacial implants. 12(1):106-112.

Aune D, Sen A, Schlesinger S, Norat T, Janszky I, Romundstad P, Tonstad S, Riboli E, Vatten LJ. 2017. Body mass index, abdominal fatness, fat mass and the risk of atrial fibrillation: A systematic review and dose–response meta-analysis of prospective studies. Springer.

Baddour LM, Wilson WR, Bayer AS, Fowler Jr VG, Tleyjeh IM, Rybak MJ, Barsic B, Lockhart PB, Gewitz MH, Levison ME. 2015. Infective endocarditis in adults: Diagnosis, antimicrobial therapy, and management of complications: A scientific statement for healthcare professionals from the american heart association. Circulation. 132(15):1435-1486.

Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, Peto R, Barnes EH, Keech A, Simes J et al. 2010. Efficacy and safety of more intensive lowering of ldl cholesterol: A meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 376(9753):1670-1681.

Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R et al. 2005. Efficacy and safety of cholesterol-lowering treatment: Prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet. 366(9493):1267-1278.

Balejo RDP, Cortelli JR, Costa FO, Cyrino RM, Aquino DR, Cogo-Müller K, Miranda TB, Moura SP, Cortelli SC. 2017. Effects of chlorhexidine preprocedural rinse on bacteremia in periodontal patients: A randomized clinical trial. J Appl Oral Sci. 25(6):586-595.

Barter PJ, Brandrup-Wognsen G, Palmer MK, Nicholls SJ. 2010. Effect of statins on hdl-c: A complex process unrelated to changes in ldl-c: Analysis of the voyager database. Journal of lipid research. 51(6):1546-1553.

Basegmez C, Yalcin S, Yalcin F, Ersanli S, Mijiritsky E. 2012. Evaluation of periimplant crevicular fluid prostaglandin e2 and matrix metalloproteinase-8 levels from health to periimplant disease status: A prospective study. Implant Dentistry. 21(4):306-310.

Bélanger M, Kozarov E, Song H, Whitlock J, Progulske-Fox A. 2012. Both the unique and repeat regions of the porphyromonas gingivalis hemagglutin a are involved in adhesion and invasion of host cells. Anaerobe. 18(1):128-134.

Belibasakis GN, Mir-Mari J, Sahrmann P, Sanz-Martin I, Schmidlin PR, Jung RE. 2016. Clinical association of spirochaetes and synergistetes with peri-implantitis. Clin Oral Implants Res. 27(6):656-661.

Bentzon JF, Otsuka F, Virmani R, Falk E. 2014. Mechanisms of plaque formation and rupture. Circ Res. 114(12):1852-1866.

Berglundh T, Armitage G, Araujo MG, Avila-Ortiz G, Blanco J, Camargo PM, Chen S, Cochran D, Derks J, Figuero E et al. 2018. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. Journal of periodontology. 89(S1):S313-S318.

Berglundh T, Gislason O, Lekholm U, Sennerby L, Lindhe J. 2004. Histopathological observations of human periimplantitis lesions. J Clin Periodontol. 31(5):341-347.

Tables of summary health statistics for u.S. Adults: 2017 national health interview survey. . 2018. Centers for Disease Control and Prevention website; [accessed MAy 7 2919]. http://www.cdc.gov/nchs/nhis/SHS/tables.htm.

Blankstein R, Budoff MJ, Shaw LJ, Goff DC, Polak JF, Lima J, Blumenthal RS, Nasir K. 2011. Predictors of coronary heart disease events among asymptomatic persons with low low-density lipoprotein cholesterol: Mesa (multi-ethnic study of atherosclerosis). Journal of the American College of Cardiology. 58(4):364-374.

Bordin S, Flemmig TF, Verardi S. 2009. Role of fibroblast populations in peri-implantitis. The International journal of oral & maxillofacial implants. 24(2):197-204.

Brodzikowska A, Górska R, Kowalski J. 2019. Interleukin-1 genotype in periodontitis. Arch Immunol Ther Exp (Warsz). 67(6):367-373.

Bruunsgaard H, Andersen-Ranberg K, Hjelmborg J, Pedersen BK, Jeune B. 2003. Elevated levels of tumor necrosis factor alpha and mortality in centenarians. Am J Med. 115(4):278-283.

Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL. 1998. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes & development. 12(9):1260-1268.

Buckley DI, Fu R, Freeman M, Rogers K, Helfand M. 2009. C-reactive protein as a risk factor for coronary heart disease: A systematic review and meta-analyses for the us preventive services task force. Annals of internal medicine. 151(7):483-495.

Bullon P, Fioroni M, Goteri G, Rubini C, Battino M. 2004. Immunohistochemical analysis of soft tissues in implants with healthy and peri-implantitis condition, and aggressive periodontitis. Clinical oral implants research. 15(5):553-559.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. 2016. Dada2: High-resolution sample inference from illumina amplicon data. Nat Methods. 13(7):581-583.

Canullo L, Peñarrocha-Oltra D, Covani U, Botticelli D, Serino G, Penarrocha M. 2016. Clinical and microbiological findings in patients with peri-implantitis: A cross-sectional study. Clin Oral Implants Res. 27(3):376-382.

Canullo L, Rossetti PH, Penarrocha D. 2015. Identification of enterococcus faecalis and pseudomonas aeruginosa on and in implants in individuals with peri-implant disease: A cross-sectional study. The International journal of oral & maxillofacial implants. 30(3):583-587.

Carcuac O, Berglundh T. 2014. Composition of human peri-implantitis and periodontitis lesions. J Dent Res. 93(11):1083-1088.

Carter SJ, Bryan DR, Neumeier WH, Glasser SP, Hunter GR. 2018. Serum tumor necrosis factor-alpha associates with myocardial oxygen demand and exercise tolerance in postmenopausal women. Int J Exerc Sci. 11(2):42-54.

Centers for Disease Control and Prevention. 2019. National center for health statistics. National health and nutrition examination survey (nhanes) public use data files.

Chamis AL, Peterson GE, Cabell CH, Corey GR, Sorrentino RA, Greenfield RA, Ryan T, Reller LB, Fowler Jr VG. 2001. Staphylococcus aureus bacteremia in patients with permanent pacemakers or implantable cardioverter-defibrillators. Circulation. 104(9):1029-1033.

Chang AY, Skirbekk VF, Tyrovolas S, Kassebaum NJ, Dieleman JL. 2019. Measuring population ageing: An analysis of the global burden of disease study 2017. The Lancet Public Health. 4(3):e159-e167.

Chapple IL, Genco R, workshop* wgotjEA. 2013. Diabetes and periodontal diseases: Consensus report of the joint efp/aap workshop on periodontitis and systemic diseases. Journal of periodontology. 84:S106-S112.

Chapple ILC, Mealey BL, Van Dyke TE, Bartold PM, Dommisch H, Eickholz P, Geisinger ML, Genco RJ, Glogauer M, Goldstein M et al. 2018. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. Journal of periodontology. 89(S1):S74-S84.

Charalampakis G, Leonhardt Å, Rabe P, Dahlén G. 2012. Clinical and microbiological characteristics of peri-implantitis cases: A retrospective multicentre study. Clin Oral Implants Res. 23(9):1045-1054.

Chen D-Y, Lin C-H, Chen Y-M, Chen H-H. 2016. Risk of atrial fibrillation or flutter associated with periodontitis: A nationwide, population-based, cohort study. PloS one. 11(10):e0165601.

Cheng R, Wu Z, Li M, Shao M, Hu T. 2020. Interleukin-1β is a potential therapeutic target for periodontitis: A narrative review. Int J Oral Sci. 12(1):2.

Chobanian A. 2003. National heart, lung, and blood institute; national high blood pressure education program coordinating committee. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. Hypertension. 42:1206-1252.

Chukkapalli SS, Velsko IM, Rivera-Kweh MF, Zheng D, Lucas AR, Kesavalu L. 2015. Polymicrobial oral infection with four periodontal bacteria orchestrates a distinct inflammatory response and atherosclerosis in apoe null mice. PLoS One. 10(11):e0143291.

Cionca N, Hashim D, Cancela J, Giannopoulou C, Mombelli A. 2016. Pro-inflammatory cytokines at zirconia implants and teeth. A cross-sectional assessment. Clinical oral investigations. 20(8):2285-2291.

Clarke MCH, Talib S, Figg NL, Bennett MR. 2010. Vascular smooth muscle cell apoptosis induces interleukin-1–directed inflammation: Effects of hyperlipidemia-mediated inhibition of phagocytosis. Circulation research. 106(2):363-372.

Conti P, Shaik-Dasthagirisaeb Y. 2015. Atherosclerosis: A chronic inflammatory disease mediated by mast cells. Cent Eur J Immunol. 40(3):380-386.

Cooper-DeHoff RM, Johnson JA. 2016. Hypertension pharmacogenomics: In search of personalized treatment approaches. Nature Reviews Nephrology. 12(2):110.

Cornelini R, Artese L, Rubini C, Fioroni M, Ferrero G, Santinelli A, Piattelli A. 2001. Vascular endothelial growth factor and microvessel density around healthy and failing dental implants. The International journal of oral & maxillofacial implants. 16(3):389-393.

Cortelli SC, Cortelli JR, Romeiro RL, Costa FO, Aquino DR, Orzechowski PR, Araújo VC, Duarte PM. 2013. Frequency of periodontal pathogens in equivalent peri-implant and periodontal clinical statuses. Archives of oral biology. 58(1):67-74.

Costa FO, Ferreira SD, Cortelli JR, Lima RPE, Cortelli SC, Cota LOM. 2019. Microbiological profile associated with peri-implant diseases in individuals with and without preventive maintenance therapy: A 5-year follow-up. Clinical oral investigations. 23(8):3161-3171.

Coumbe AG, Pritzker MR, Duprez DA. 2014. Cardiovascular risk and psoriasis: Beyond the traditional risk factors. Am J Med. 127(1):12-18.

Cozlea DL, Farcas DM, Nagy A, Keresztesi AA, Tifrea R, Cozlea L, Carașca E. 2013. The impact of c reactive protein on global cardiovascular risk on patients with coronary artery disease. Curr Health Sci J. 39(4):225-231.

Csiszar A, Labinskyy N, Smith K, Rivera A, Orosz Z, Ungvari Z. 2007. Vasculoprotective effects of anti-tumor necrosis factor-α treatment in aging. The American journal of pathology. 170(1):388-398.

Dalago HR, Schuldt Filho G, Rodrigues MA, Renvert S, Bianchini MA. 2017. Risk indicators for peri-implantitis. A cross-sectional study with 916 implants. Clin Oral Implants Res. 28(2):144-150.

Danaei G, Ding EL, Mozaffarian D, Taylor B, Rehm J, Murray CJ, Ezzati M. 2009. The preventable causes of death in the united states: Comparative risk assessment of dietary, lifestyle, and metabolic risk factors. PLoS Med. 6(4):e1000058.

Danesh J, Collins R, Peto R. 1997. Chronic infections and coronary heart disease: Is there a link? Lancet. 350(9075):430-436.

Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, Kostis JB, Wilson AC, Folsom AR, Wu K, Benderly M et al. 2005. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: An individual participant meta-analysis. Jama. 294(14):1799-1809.

Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, Gallimore JR, Pepys MB. 2000. Low grade inflammation and coronary heart disease: Prospective study and updated meta-analyses. BMJ (Clinical research ed). 321(7255):199-204.

de Boer SP, Cheng JM, Rangé H, Garcia-Garcia HM, Heo JH, Akkerhuis KM, Meilhac O, Cosler G, Pussinen PJ, van Geuns R-J. 2014. Antibodies to periodontal pathogens are associated with coronary plaque remodeling but not with vulnerability or burden. Atherosclerosis. 237(1):84-91.

De Boever AL, De Boever JA. 2006. Early colonization of non-submerged dental implants in patients with a history of advanced aggressive periodontitis. Clinical Oral Implants Research. 17(1):8-17.

de Mendonça AC, Santos VR, César-Neto JB, Duarte PM. 2009. Tumor necrosis factor-alpha levels after surgical anti-infective mechanical therapy for peri-implantitis: A 12-month follow-up. Journal of periodontology. 80(4):693-699.

de Rooij SR, Nijpels G, Nilsson PM, Nolan JJ, Gabriel R, Bobbioni-Harsch E, Mingrone G, Dekker JM. 2009. Low-grade chronic inflammation in the relationship between insulin sensitivity and cardiovascular disease (risc) population: Associations with insulin resistance and cardiometabolic risk profile. Diabetes Care. 32(7):1295-1301.

de Waal YC, Eijsbouts HV, Winkel EG, van Winkelhoff AJ. 2017. Microbial characteristics of peri-implantitis: A case-control study. Journal of periodontology. 88(2):209-217.

de Waal YC, Winkel EG, Meijer HJ, Raghoebar GM, van Winkelhoff AJ. 2014. Differences in peri-implant microflora between fully and partially edentulous patients: A systematic review. Journal of periodontology. 85(1):68-82.

Dehghan A, Dupuis J, Barbalic M, Bis JC, Eiriksdottir G, Lu C, Pellikka N, Wallaschofski H, Kettunen J, Henneman P. 2011. Meta-analysis of genome-wide association studies in> 80 000 subjects identifies multiple loci for c-reactive protein levels. Circulation. 123(7):731-738.

Derks J, Schaller D, Håkansson J, Wennström J, Tomasi C, Berglundh T. 2016. Effectiveness of implant therapy analyzed in a swedish population: Prevalence of peri-implantitis. Journal of dental research. 95(1):43-49.

Derks J, Tomasi C. 2015. Peri-implant health and disease. A systematic review of current epidemiology. Journal of clinical periodontology. 42:S158-S171.

Desvarieux M, Demmer RT, Rundek T, Boden-Albala B, Jacobs DR, Jr., Sacco RL, Papapanou PN. 2005. Periodontal microbiota and carotid intima-media thickness: The oral infections and vascular disease epidemiology study (invest). Circulation. 111(5):576-582.

Detrano R, Guerci AD, Carr JJ, Bild DE, Burke G, Folsom AR, Liu K, Shea S, Szklo M, Bluemke DA et al. 2008. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. New England Journal of Medicine. 358(13):1336-1345.

Dhore CR, Cleutjens JP, Lutgens E, Cleutjens KB, Geusens PP, Kitslaar PJ, Tordoir JH, Spronk HM, Vermeer C, Daemen MJ. 2001. Differential expression of bone matrix regulatory proteins in human atherosclerotic plaques. Arterioscler Thromb Vasc Biol. 21(12):1998-2003.

Dietrich T, Sharma P, Walter C, Weston P, Beck J. 2013. The epidemiological evidence behind the association between periodontitis and incident atherosclerotic cardiovascular disease. J Clin Periodontol. 40 Suppl 14:S70-84.

Dinarello CA. 2011. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood. 117(14):3720-3732.

Duarte P, Serrão C, Miranda T, Zanatta L, Bastos M, Faveri M, Figueiredo L, Feres M. 2016. Could cytokine levels in the peri-implant crevicular fluid be used to distinguish between healthy implants and implants with peri-implantitis? A systematic review. Journal of periodontal research. 51(6):689-698.

Duarte PM, de Mendonça AC, Máximo MB, Santos VR, Bastos MF, Nociti Júnior FH. 2009a. Differential cytokine expressions affect the severity of peri-implant disease. Clin Oral Implants Res. 20(5):514-520.

Duarte PM, de Mendonça AC, Máximo MBB, Santos VR, Bastos MF, Nociti Jr FH. 2009b. Effect of anti-infective mechanical therapy on clinical parameters and cytokine levels in human peri-implant diseases. Journal of periodontology. 80(2):234-243.

Duzenli AE, Dwyer J, Carey J. 2017. Haemophilus parainfluenzae endocarditis associated with maxillary sinusitis and complicated by cerebral emboli in a young man. J Investig Med High Impact Case Rep. 5(2):2324709617704003-2324709617704003.

Ebersole JL, Dawson DA, 3rd, Emecen Huja P, Pandruvada S, Basu A, Nguyen L, Zhang Y, Gonzalez OA. 2018. Age and periodontal health - immunological view. Current oral health reports. 5(4):229-241.

Eckel N, Meidtner K, Kalle-Uhlmann T, Stefan N, Schulze MB. 2016. Metabolically healthy obesity and cardiovascular events: A systematic review and meta-analysis. European journal of preventive cardiology. 23(9):956-966.

Elkaim R, Dahan M, Kocgozlu L, Werner S, Kanter D, Kretz J, Tenenbaum H. 2008. Prevalence of periodontal pathogens in subgingival lesions, atherosclerotic plaques and healthy blood vessels: A preliminary study. Journal of periodontal research. 43(2):224-231.

Elliott P, Chambers JC, Zhang W, Clarke R, Hopewell JC, Peden JF, Erdmann J, Braund P, Engert JC, Bennett D. 2009. Genetic loci associated with c-reactive protein levels and risk of coronary heart disease. Jama. 302(1):37-48.

Emerging Risk Factors Collaboration. 2010. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. The Lancet. 375(9733):2215-2222.

Eriksson JK, Jacobsson L, Bengtsson K, Askling J. 2017. Is ankylosing spondylitis a risk factor for cardiovascular disease, and how do these risks compare with those in rheumatoid arthritis? Ann Rheum Dis. 76(2):364-370.

Faot F, Nascimento GG, Bielemann AM, Campão TD, Leite FR, Quirynen M. 2015. Can peri-implant crevicular fluid assist in the diagnosis of peri-implantitis? A systematic review and meta-analysis. Journal of periodontology. 86(5):631-645.

Feingold and Grunfeld. 2019. The effect of inflammation and infection on lipids and lipoproteins. . [Updated 2019 Jan 2018]. In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext [Internet].

Feng W, Chen G, Cai D, Zhao S, Cheng J, Shen H. 2017. Inflammatory bowel disease and risk of ischemic heart disease: An updated meta-analysis of cohort studies. J Am Heart Assoc. 6(8).

Fernandes CP, Oliveira FAF, de Barros Silva PG, Alves APNN, Mota MRL, Montenegro RC, Burbano RMR, Seabra AD, Lobo Filho JG, Lima DLF. 2014. Molecular analysis of oral bacteria in dental biofilm and atherosclerotic plaques of patients with vascular disease. International journal of cardiology. 174(3):710-712.

Fernández-Friera L, Fuster V, López-Melgar B, Oliva B, García-Ruiz JM, Mendiguren J, Bueno H, Pocock S, Ibáñez B, Fernández-Ortiz A. 2017. Normal ldl-cholesterol levels are associated with subclinical atherosclerosis in the absence of risk factors. Journal of the American College of Cardiology. 70(24):2979-2991.

Figuero E, Lindahl C, Marín MJ, Renvert S, Herrera D, Ohlsson O, Wetterling T, Sanz M. 2014. Quantification of periodontal pathogens in vascular, blood, and subgingival samples from patients with peripheral arterial disease or abdominal aortic aneurysms. Journal of periodontology. 85(9):1182-1193.

Figuero E, Sánchez-Beltrán M, Cuesta-Frechoso S, Tejerina JM, del Castro JA, Gutiérrez JM, Herrera D, Sanz M. 2011. Detection of periodontal bacteria in atheromatous plaque by nested polymerase chain reaction. Journal of periodontology. 82(10):1469-1477.

Fonarow GC, Calitz C, Arena R, Baase C, Isaac FW, Lloyd-Jones D, Peterson ED, Pronk N, Sanchez E, Terry PE. 2015. Workplace wellness recognition for optimizing workplace health: A presidential advisory from the american heart association. Circulation. 131(20):e480-e497.

Ford ES, Greenlund KJ, Hong Y. 2012. Ideal cardiovascular health and mortality from all causes and diseases of the circulatory system among adults in the united states. Circulation. 125(8):987-995.

Ford P, Gemmell E, Chan A, Carter C, Walker P, Bird P, West M, Cullinan M, Seymour G. 2006. Inflammation, heat shock proteins and periodontal pathogens in atherosclerosis: An immunohistologic study. Oral microbiology and immunology. 21(4):206-211.

Forouzanfar MH, Afshin A, Alexander LT, Anderson HR, Bhutta ZA, Biryukov S, Brauer M, Burnett R, Cercy K, Charlson FJ. 2016. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: A systematic analysis for the global burden of disease study 2015. The lancet. 388(10053):1659-1724.

Fowler VG, Miro JM, Hoen B, Cabell CH, Abrutyn E, Rubinstein E, Corey GR, Spelman D, Bradley SF, Barsic B. 2005. Staphylococcus aureus endocarditis: A consequence of medical progress. Jama. 293(24):3012-3021.

Frostegård J. 2013. Immunity, atherosclerosis and cardiovascular disease. BMC Medicine. 11(1):117.

Fryar CD, Chen TC, Li X. 2012. Prevalence of uncontrolled risk factors for cardiovascular disease: United states, 1999-2010. NCHS Data Brief. (103):1-8.

Fürst MM, Salvi GE, Lang NP, Persson GR. 2007. Bacterial colonization immediately after installation on oral titanium implants. Clin Oral Implants Res. 18(4):501-508.

GBD Risk Factor Collaborators. 2018. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: A systematic analysis for the global burden of disease study 2017. Lancet (London, England). 392(10159):1923.

Ghassib I, Chen Z, Zhu J, Wang HL. 2019. Use of il-1 β, il-6, tnf-α, and mmp-8 biomarkers to distinguish peri-implant diseases: A systematic review and meta-analysis. Clinical implant dentistry and related research. 21(1):190-207.

Ghighi M, Llorens A, Baroukh B, Chaussain C, Bouchard P, Gosset M. 2018. Differences between inflammatory and catabolic mediators of peri-implantitis and periodontitis lesions following initial mechanical therapy: An exploratory study. J Periodontal Res. 53(1):29-39.

Giannopoulou C, Kamma JJ, Mombelli A. 2003. Effect of inflammation, smoking and stress on gingival crevicular fluid cytokine level. J Clin Periodontol. 30(2):145-153.

Goetzel RZ, Henke RM, Head MA, Benevent R, Calitz C. 2017. Workplace programs, policies, and environmental supports to prevent cardiovascular disease. Health Affairs. 36(2):229-236.

Golub LM, Lee HM. 2020. Periodontal therapeutics: Current host-modulation agents and future directions. Periodontol 2000. 82(1):186-204.

Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, Foster E, Hlatky MA, Hodgson JM, Kushner FG et al. 2010. 2010 accf/aha guideline for assessment of cardiovascular risk in asymptomatic adults: A report of the american college of cardiology foundation/american heart association task force on practice guidelines. J Am Coll Cardiol. 56(25):e50-103.

Grundy SM, Brewer HB, Cleeman JI, Smith SC, Lenfant C. 2004. Definition of metabolic syndrome. Circulation. 109(3):433-438.

Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, Braun LT, Ferranti Sd, Faiella-Tommasino J, Forman DE et al. 2019. 2018 aha/acc/aacvpr/aapa/abc/acpm/ada/ags/apha/aspc/nla/pcna guideline on the management of blood cholesterol: A report of the american college of cardiology/american heart association task force on clinical practice guidelines. Circulation. 139(25):e1082-e1143.

Guo F, Moellering DR, Garvey WT. 2014. The progression of cardiometabolic disease: Validation of a new cardiometabolic disease staging system applicable to obesity. Obesity (Silver Spring). 22(1):110-118.

Gürlek Ö, Gümüş P, Nile CJ, Lappin DF, Buduneli N. 2017. Biomarkers and bacteria around implants and natural teeth in the same individuals. Journal of periodontology. 88(8):752-761.

Hales CM, Fryar CD, Carroll MD, Freedman DS, Ogden CL. 2018. Trends in obesity and severe obesity prevalence in us youth and adults by sex and age, 2007-2008 to 2015-2016. Jama. 319(16):1723-1725.

Hallström H, Persson GR, Lindgren S, Olofsson M, Renvert S. 2012. Systemic antibiotics and debridement of peri-implant mucositis. A randomized clinical trial. Journal of clinical periodontology. 39(6):574-581.

Hamp SE, Nyman S, Lindhe J. 1975. Periodontal treatment of multirooted teeth. Results after 5 years. J Clin Periodontol. 2(3):126-135.

Han YW. 2015. Fusobacterium nucleatum: A commensal-turned pathogen. Curr Opin Microbiol. 23:141-147.

Han YW, Wang X. 2013. Mobile microbiome: Oral bacteria in extra-oral infections and inflammation. J Dent Res. 92(6):485-491.

Haque S, Mirjafari H, Bruce IN. 2008. Atherosclerosis in rheumatoid arthritis and systemic lupus erythematosus. Curr Opin Lipidol. 19(4):338-343.

Harris LG, Meredith DO, Eschbach L, Richards RG. 2007. Staphylococcus aureus adhesion to standard micro-rough and electropolished implant materials. J Mater Sci Mater Med. 18(6):1151-1156.

Hayashi C, Viereck J, Hua N, Phinikaridou A, Madrigal AG, Gibson III FC, Hamilton JA, Genco CA. 2011. Porphyromonas gingivalis accelerates inflammatory atherosclerosis in the innominate artery of apoe deficient mice. Atherosclerosis. 215(1):52-59.

Heasman PA, Lauffart BL, Preshaw PM. 1998. Crevicular fluid prostaglandin e2 levels in periodontitis-resistant and periodontitis-susceptible adults. J Clin Periodontol. 25(12):1003-1007.

Heitz-Mayfield LJ, Aaboe M, Araujo M, Carrión JB, Cavalcanti R, Cionca N, Cochran D, Darby I, Funakoshi E, Gierthmuehlen PC et al. 2018. Group 4 iti consensus report: Risks and biologic complications associated with implant dentistry. Clinical Oral Implants Research. 29(S16):351-358.

Heitz-Mayfield LJ, Lang NP. 2010. Comparative biology of chronic and aggressive periodontitis vs. Peri-implantitis. Periodontol 2000. 53:167-181.

Heitz-Mayfield LJ, Needleman I, Salvi GE, Pjetursson BE. 2014. Consensus statements and clinical recommendations for prevention and management of biologic and technical implant complications. The International journal of oral & maxillofacial implants. 29 Suppl:346-350.

Heitz-Mayfield LJA, Heitz F, Lang NP. 2020. Implant disease risk assessment idra-a tool for preventing peri-implant disease. Clin Oral Implants Res. 31(4):397-403.

Heitz-Mayfield LJA, Salvi GE. 2018. Peri-implant mucositis. Journal of periodontology. 89(S1):S257-S266.

Hernández-Rodríguez J, Segarra M, Vilardell C, Sánchez M, García-Martínez A, Esteban MJ, Queralt C, Grau JM, Urbano-Márquez A, Palacín A et al. 2003. Tissue production of pro-inflammatory cytokines (il-1β, tnfα and il-6) correlates with the intensity of the systemic inflammatory response and with corticosteroid requirements in giant-cell arteritis. Rheumatology. 43(3):294-301.

Herrera D, Molina A, Buhlin K, Klinge B. 2020. Periodontal diseases and association with atherosclerotic disease. Periodontol 2000. 83(1):66-89.

Hippisley-Cox J, Coupland C, Brindle P. 2017. Development and validation of qrisk3 risk prediction algorithms to estimate future risk of cardiovascular disease: Prospective cohort study. bmj. 357:j2099.

Hoerr V, Franz M, Pletz MW, Diab M, Niemann S, Faber C, Doenst T, Schulze PC, Deinhardt-Emmer S, Löffler B. 2018. S. Aureus endocarditis: Clinical aspects and experimental approaches. Int J Med Microbiol. 308(6):640-652.

Hofbauer LC, Schoppet M. 2004. Clinical implications of the osteoprotegerin/rankl/rank system for bone and vascular diseases. Jama. 292(4):490-495.

Hofbauer LC, Shui C, Riggs BL, Dunstan CR, Spelsberg TC, O'Brien T, Khosla S. 2001. Effects of immunosuppressants on receptor activator of nf-κb ligand and osteoprotegerin production by human osteoblastic and coronary artery smooth muscle cells. Biochemical and biophysical research communications. 280(1):334-339.

Hosomi N, Aoki S, Matsuo K, Deguchi K, Masugata H, Murao K, Ichihara N, Ohyama H, Dobashi H, Nezu T. 2012. Association of serum anti-periodontal pathogen antibody with ischemic stroke. Cerebrovascular Diseases. 34(5-6):385-392.

Jankovic S, Aleksic Z, Dimitrijevic B, Lekovic V, Camargo P, Kenney B. 2011. Prevalence of human cytomegalovirus and epstein-barr virus in subgingival plaque at peri-implantitis, mucositis and healthy sites. A pilot study. International journal of oral and maxillofacial surgery. 40(3):271-276.

Jepsen S, Berglundh T, Genco R, Aass AM, Demirel K, Derks J, Figuero E, Giovannoli JL, Goldstein M, Lambert F. 2015. Primary prevention of peri-implantitis: Managing peri-implant mucositis. Journal of clinical periodontology. 42:S152-S157.

Jono S, Ikari Y, Shioi A, Mori K, Miki T, Hara K, Nishizawa Y. 2002. Serum osteoprotegerin levels are associated with the presence and severity of coronary artery disease. Circulation. 106(10):1192-1194.

Joseph P, Leong D, McKee M, Anand SS, Schwalm J-D, Teo K, Mente A, Yusuf S. 2017. Reducing the global burden of cardiovascular disease, part 1: The epidemiology and risk factors. Circulation research. 121(6):677-694.

Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. 1987. Fibrinogen and risk of cardiovascular disease. The framingham study. Jama. 258(9):1183-1186.

Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J. 2010. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: An individual participant meta-analysis. Lancet. 375(9709):132-140.

Katz J, Chaushu G, Sharabi Y. 2001. On the association between hypercholesterolemia, cardiovascular disease and severe periodontal disease. Journal of clinical periodontology. 28(9):865-868.

Kaushal S, Singh AK, Lal N, Das SK, Mahdi AA. 2019. Effect of periodontal therapy on disease activity in patients of rheumatoid arthritis with chronic periodontitis. Journal of oral biology and craniofacial research. 9(2):128-132.

Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C. 2004. Effects of infection and inflammation on lipid and lipoprotein metabolism: Mechanisms and consequences to the host. J Lipid Res. 45(7):1169-1196.

Kinlay S. 2007. Low-density lipoprotein-dependent and-independent effects of cholesterol-lowering therapies on c-reactive protein: A meta-analysis. Journal of the American College of Cardiology. 49(20):2003-2009.

Kivelä-Rajamäki M, Maisi P, Srinivas R, Tervahartiala T, Teronen O, Husa V, Salo T, Sorsa T. 2003. Levels and molecular forms of mmp-7 (matrilysin-1) and mmp-8 (collagenase-2) in diseased human peri-implant sulcular fluid. J Periodontal Res. 38(6):583-590.

Klein S, Burke LE, Bray GA, Blair S, Allison DB, Pi-Sunyer X, Hong Y, Eckel RH. 2004. Clinical implications of obesity with specific focus on cardiovascular disease: A statement for professionals from the american heart association council on nutrition, physical activity, and metabolism: Endorsed by the american college of cardiology foundation. Circulation. 110(18):2952-2967.

Koenig W. 2018. Low-grade inflammation modifies cardiovascular risk even at very low ldl-c levels. Circulation. 138(2):150-153.

Koike T, Kitajima S, Yu Y, Nishijima K, Zhang J, Ozaki Y, Morimoto M, Watanabe T, Bhakdi S, Asada Y. 2009. Human c-reactive protein does not promote atherosclerosis in transgenic rabbits. Circulation. 120(21):2088.

Koldsland OC, Scheie AA, Aass AM. 2010. Prevalence of peri-implantitis related to severity of the disease with different degrees of bone loss. Journal of periodontology. 81(2):231-238.

Køllgaard T, Enevold C, Bendtzen K, Hansen PR, Givskov M, Holmstrup P, Nielsen CH. 2017. Cholesterol crystals enhance tlr2-and tlr4-mediated pro-inflammatory cytokine responses of monocytes to the proatherogenic oral bacterium porphyromonas gingivalis. PLoS One. 12(2):e0172773.

Konttinen YT, Ma J, Lappalainen R, Laine P, Kitti U, Santavirta S, Teronen O. 2006. Immunohistochemical evaluation of inflammatory mediators in failing implants. International Journal of Periodontics & Restorative Dentistry. 26(2).

Koyanagi T, Sakamoto M, Takeuchi Y, Maruyama N, Ohkuma M, Izumi Y. 2013. Comprehensive microbiological findings in peri-implantitis and periodontitis. Journal of clinical periodontology. 40(3):218-226.

Kozarov EV, Dorn BR, Shelburne CE, Dunn WA, Jr., Progulske-Fox A. 2005. Human atherosclerotic plaque contains viable invasive actinobacillus actinomycetemcomitans and porphyromonas gingivalis. Arterioscler Thromb Vasc Biol. 25(3):e17-18.

Krabbe KS, Pedersen M, Bruunsgaard H. 2004. Inflammatory mediators in the elderly. Exp Gerontol. 39(5):687-699.

Kronström M, Svenson B, Hellman M, Persson GR. 2001. Early implant failures in patients treated with bränemark system titanium dental implants: A retrospective study. International Journal of Oral & Maxillofacial Implants. 16(2).

Kronström M, Svensson B, Erickson E, Houston L, Braham P, Persson GR. 2000. Humoral immunity host factors in subjects with failing or successful titanium dental implants. Journal of clinical periodontology. 27(12):875-882.

Kyu HH, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, Abbastabar H, Abd-Allah F, Abdela J, Abdelalim A. 2018. Global, regional, and national disability-adjusted life-years (dalys) for 359 diseases and injuries and healthy life expectancy (hale) for 195 countries and territories, 1990–2017: A systematic analysis for the global burden of disease study 2017. The Lancet. 392(10159):1859-1922.

Lachmann S, Stehberger A, Axmann D, Weber H. 2013. The peri-implant health in patients attending an annual recall program. A clinical and microbiological study in 74 patients from the tübingen implant registry. Clinical oral implants research. 24(12):1300-1309.

Lafaurie GI, Sabogal MA, Castillo DM, Rincón MV, Gómez LA, Lesmes YA, Chambrone L. 2017. Microbiome and microbial biofilm profiles of peri-implantitis: A systematic review. Journal of periodontology. 88(10):1066-1089.

Laine ML, Leonhardt A, Roos-Jansåker AM, Peña AS, van Winkelhoff AJ, Winkel EG, Renvert S. 2006. Il-1rn gene polymorphism is associated with peri-implantitis. Clin Oral Implants Res. 17(4):380-385.

Lang NP, Bartold PM. 2018. Periodontal health. Journal of periodontology. 89(S1):S9-S16.

Lang NP, Berglundh T, Periodontology WGotSEWo. 2011. Periimplant diseases: Where are we now?– consensus of the seventh european workshop on periodontology. Journal of clinical periodontology. 38:178-181.

Le JM, Vilcek J. 1989. Interleukin 6: A multifunctional cytokine regulating immune reactions and the acute phase protein response. Lab Invest. 61(6):588-602.

Lee S, Im A, Burm E, Ha M. 2018. Association between periodontitis and blood lipid levels in a korean population. Journal of periodontology. 89(1):28-35.

Leira Y, Seoane J, Blanco M, Rodriguez-Yanez M, Takkouche B, Blanco J, Castillo J. 2017. Association between periodontitis and ischemic stroke: A systematic review and meta-analysis. Springer.

Leon BM, Maddox TM. 2015. Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. World J Diabetes. 6(13):1246-1258.

Leonhardt A, Renvert S, Dahlén G. 1999. Microbial findings at failing implants. Clin Oral Implants Res. 10(5):339-345.

Libby P. 2017. Interleukin-1 beta as a target for atherosclerosis therapy: Biological basis of cantos and beyond. Journal of the American College of Cardiology. 70(18):2278-2289.

Libby P, Okamoto Y, Rocha VZ, Folco E. 2010. Inflammation in atherosclerosis: Transition from theory to practice. Circulation journal. 74(2):213-220.

Libby P, Ridker PM, Hansson GK. 2009. Inflammation in atherosclerosis: From pathophysiology to practice. J Am Coll Cardiol. 54(23):2129-2138.

Liccardo D, Cannavo A, Spagnuolo G, Ferrara N, Cittadini A, Rengo C, Rengo G. 2019. Periodontal disease: A risk factor for diabetes and cardiovascular disease. Int J Mol Sci. 20(6):1414.

Lieb W, Gona P, Larson MG, Massaro JM, Lipinska I, Keaney Jr JF, Rong J, Corey D, Hoffmann U, Fox CS. 2010. Biomarkers of the osteoprotegerin pathway: Clinical correlates, subclinical disease, incident cardiovascular disease, and mortality. Arteriosclerosis, thrombosis, and vascular biology. 30(9):1849-1854.

Linden GJ, Linden K, Yarnell J, Evans A, Kee F, Patterson CC. 2012. All-cause mortality and periodontitis in 60–70-year-old men: A prospective cohort study. Journal of clinical periodontology. 39(10):940-946.

Linden GJ, Lyons A, Scannapieco FA. 2013. Periodontal systemic associations: Review of the evidence. Journal of periodontology. 84:S8-S19.

Liu F, Wang Y, Xu J, Liu F, Hu R, Deng H. 2016a. Effects of porphyromonas gingivalis lipopolysaccharide on the expression of key genes involved in cholesterol metabolism in macrophages. Archives of Medical Science: AMS. 12(5):959.

Liu L, Simon B, Shi J, Mallhi AK, Eisen HJ. 2016b. Impact of diabetes mellitus on risk of cardiovascular disease and all-cause mortality: Evidence on health outcomes and antidiabetic treatment in united states adults. World J Diabetes. 7(18):449.

Lleo A, Selmi C, Invernizzi P, Podda M, Gershwin ME. 2008. The consequences of apoptosis in autoimmunity. J Autoimmun. 31(3):257-262.

Lloyd-Jones D. 2010. American heart association strategic planning task force and statistics committee. Defining and setting national goats for cardiovascular health promotion and disease reduction: The american heart association's strategic impact goal through 2020 and beyond. Circulation. 121:586-613.

Lloyd-Jones DM, Braun LT, Ndumele CE, Smith SC, Jr., Sperling LS, Virani SS, Blumenthal RS. 2019. Use of risk assessment tools to guide decision-making in the primary prevention of atherosclerotic cardiovascular disease: A special report from the american heart association and american college of cardiology. Circulation. 139(25):e1162-e1177.

Lönn J, Ljunggren S, Klarström-Engström K, Demirel I, Bengtsson T, Karlsson H. 2018. Lipoprotein modifications by gingipains of porphyromonas gingivalis. Journal of periodontal research. 53(3):403-413.

Lopez-Candales A, Hernández Burgos PM, Hernandez-Suarez DF, Harris D. 2017. Linking chronic inflammation with cardiovascular disease: From normal aging to the metabolic syndrome. J Nat Sci. 3(4):e341.

Ma J, Hennekens CH, Ridker PM, Stampfer MJ. 1999. A prospective study of fibrinogen and risk of myocardial infarction in the physicians' health study. J Am Coll Cardiol. 33(5):1347-1352.

Ma J, Kitti U, Teronen O, Sorsa T, Husa V, Laine P, Rönkä H, Salo T, Lindqvist C, Konttinen YT. 2000. Collagenases in different categories of peri-implant vertical bone loss. J Dent Res. 79(11):1870-1873.

Mahendra J, Little Mahendra JF, Romanos G. 2013. Prevelance of periodontopathogenic bacteria in subgingival biofilm and atherosclerotic plaques of patients undergoing coronary revascularization surgery. Journal of Indian Society of Periodontology. 17(6):719.

Maskarinec SA, Thaden JT, Cyr DD, Ruffin F, Souli M, Fowler VG. 2017. The risk of cardiac device-related infection in bacteremic patients is species specific: Results of a 12-year prospective cohort. Open Forum Infect Dis. 4(3):ofx132.

Matheus AS, Tannus LR, Cobas RA, Palma CC, Negrato CA, Gomes MB. 2013. Impact of diabetes on cardiovascular disease: An update. Int J Hypertens. 2013:653789.

McCormack MG, Smith AJ, Akram AN, Jackson M, Robertson D, Edwards G. 2015. Staphylococcus aureus and the oral cavity: An overlooked source of carriage and infection? Am J Infect Control. 43(1):35-37.

McInnes IB, Thompson L, Giles JT, Bathon JM, Salmon JE, Beaulieu AD, Codding CE, Carlson TH, Delles C, Lee JS et al. 2015. Effect of interleukin-6 receptor blockade on surrogates of vascular risk in rheumatoid arthritis: Measure, a randomised, placebo-controlled study. Ann Rheum Dis. 74(4):694-702.

McTaggart F, Jones P. 2008. Effects of statins on high-density lipoproteins: A potential contribution to cardiovascular benefit. Cardiovasc Drugs Ther. 22(4):321-338.

Melo RF, Lopes BM, Shibli JA, Marcantonio E, Jr., Marcantonio RA, Galli GM. 2012. Interleukin-1β and interleukin-6 expression and gene polymorphisms in subjects with peri-implant disease. Clinical implant dentistry and related research. 14(6):905-914.

Mi Y, Yan S, Lu Y, Liang Y, Li C. 2016. Venous thromboembolism has the same risk factors as atherosclerosis: A prisma-compliant systemic review and meta-analysis. Medicine. 95(32).

Miro JM, Anguera I, Cabell CH, Chen AY, Stafford JA, Corey GR, Olaison L, Eykyn S, Hoen B, Abrutyn E et al. 2005. Staphylococcus aureus native valve infective endocarditis: Report of 566 episodes from the international collaboration on endocarditis merged database. Clin Infect Dis. 41(4):507-514.

Mombelli A. 2002. Microbiology and antimicrobial therapy of peri-implantitis. Periodontology 2000. 28(1):177-189.

Mombelli A, Décaillet F. 2011. The characteristics of biofilms in peri-implant disease. Journal of clinical periodontology. 38:203-213.

Mombelli A, van Oosten MA, Schurch E, Jr., Land NP. 1987. The microbiota associated with successful or failing osseointegrated titanium implants. Oral Microbiol Immunol. 2(4):145-151.

Mongraw-Chaffin M, Foster MC, Anderson CA, Burke GL, Haq N, Kalyani RR, Ouyang P, Sibley CT, Tracy R, Woodward M. 2018. Metabolically healthy obesity, transition to metabolic syndrome, and cardiovascular risk. Journal of the American College of Cardiology. 71(17):1857-1865.

Moreau KL, Deane KD, Meditz AL, Kohrt WM. 2013. Tumor necrosis factor-α inhibition improves endothelial function and decreases arterial stiffness in estrogen-deficient postmenopausal women. Atherosclerosis. 230(2):390-396.

Moreau KL, Gavin KM, Plum AE, Seals DR. 2006. Oxidative stress explains differences in large elastic artery compliance between sedentary and habitually exercising postmenopausal women. Menopause. 13(6):951-958.

Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, Rinfret S, Schiffrin EL, Eisenberg MJ. 2010. The metabolic syndrome and cardiovascular risk: A systematic review and meta-analysis. Journal of the American College of Cardiology. 56(14):1113-1132.

Mozaffari K, Bakhshandeh H, Soudi H. 2014. Bacteriologic profile of pericardial infections after cardiac surgery: Study in an iranian cardiovascular tertiary care center. Research in cardiovascular medicine. 3(3).

Murphy SL, Xu J, Kochanek KD, Arias E. 2018. Mortality in the united states, 2017. NCHS Data Brief. (328):1-8.

Mustapha IZ, Debrey S, Oladubu M, Ugarte R. 2007. Markers of systemic bacterial exposure in periodontal disease and cardiovascular disease risk: A systematic review and meta-analysis. Journal of periodontology. 78(12):2289-2302.

National health and nutrition examination survey (nhanes) public use data files. . 2017. Centers for Disease Control and Prevention Website; [accessed April 2019]. https://www.cdc.gov/nchs/nhanes/.

Navar-Boggan AM, Peterson ED, D'Agostino Sr RB, Neely B, Sniderman AD, Pencina MJ. 2015. Hyperlipidemia in early adulthood increases long-term risk of coronary heart disease. Circulation. 131(5):451-458.

Nissen SE, Tuzcu EM, Schoenhagen P, Crowe T, Sasiela WJ, Tsai J, Orazem J, Magorien RD, O'Shaughnessy C, Ganz P. 2005. Statin therapy, ldl cholesterol, c-reactive protein, and coronary artery disease. New England Journal of Medicine. 352(1):29-38.

Nomura T, Ishii A, Oishi Y, Kohma H, Hara K. 1998. Tissue inhibitors of metalloproteinases level and collagenase activity in gingival crevicular fluid: The relevance to periodontal diseases. Oral Dis. 4(4):231-240.

Nomura T, Ishii A, Shimizu H, Taguchi N, Yoshie H, Kusakari H, Hara K. 2000. Tissue inhibitor of metalloproteinases-1, matrix metalloproteinases-1 and -8, and collagenase activity levels in peri-implant crevicular fluid after implantation. Clin Oral Implants Res. 11(5):430-440.

O'Donnell MJ, Chin SL, Rangarajan S, Xavier D, Liu L, Zhang H, Rao-Melacini P, Zhang X, Pais P, Agapay S. 2016. Global and regional effects of potentially modifiable risk factors associated with acute stroke in 32 countries (interstroke): A case-control study. The lancet. 388(10046):761-775.

Offenbacher S, Beck JD, Moss K, Mendoza L, Paquette DW, Barrow DA, Couper DJ, Stewart DD, Falkner KL, Graham SP. 2009. Results from the periodontitis and vascular events (pave) study: A pilot multicentered, randomized, controlled trial to study effects of periodontal therapy in a secondary prevention model of cardiovascular disease. Journal of periodontology. 80(2):190-201.

Offenbacher S, Heasman PA, Collins JG. 1993. Modulation of host pge2 secretion as a determinant of periodontal disease expression. Journal of periodontology. 64(5 Suppl):432-444.

Ogdie A, Yu Y, Haynes K, Love TJ, Maliha S, Jiang Y, Troxel AB, Hennessy S, Kimmel SE, Margolis DJ et al. 2015. Risk of major cardiovascular events in patients with psoriatic arthritis, psoriasis and rheumatoid arthritis: A population-based cohort study. Ann Rheum Dis. 74(2):326-332.

Orlandi M, Graziani F, D'Aiuto F. 2020. Periodontal therapy and cardiovascular risk. Periodontol 2000. 83(1):107-124.

Ott SJ, El Mokhtari NE, Musfeldt M, Hellmig S, Freitag S, Rehman A, Kuhbacher T, Nikolaus S, Namsolleck P, Blaut M. 2006. Detection of diverse bacterial signatures in atherosclerotic lesions of patients with coronary heart disease. Circulation. 113(7):929-937.

Özkalaycı F, Gülmez Ö, Uğur-Altun B, Pandi-Perumal SR, Altun A. 2018. The role of osteoprotegerin as a cardioprotective versus reactive inflammatory marker: The chicken or the egg paradox. Balkan Med J. 35(3):225-232.

Padial-Molina M, López-Martínez J, O'Valle F, Galindo-Moreno P. 2016. Microbial profiles and detection techniques in peri-implant diseases: A systematic review. J Oral Maxillofac Res. 7(3):e10.

Palmieri V, Celentano A, Roman MJ, de Simone G, Best L, Lewis MR, Robbins DC, Fabsitz RR, Howard BV, Devereux RB. 2003. Relation of fibrinogen to cardiovascular events is independent of preclinical cardiovascular disease: The strong heart study. American heart journal. 145(3):467-474.

Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, Garcia R, Giannobile WV, Graziani F et al. 2018. Periodontitis: Consensus report of workgroup 2 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. Journal of periodontology. 89(S1):S173-S182.

Payne JB, Reinhardt RA, Masada MP, DuBois LM, Allison AC. 1993. Gingival crevicular fluid il-8: Correlation with local il-1 beta levels and patient estrogen status. J Periodontal Res. 28(6 Pt 1):451-453.

Pérez-Chaparro PJ, Duarte PM, Shibli JA, Montenegro S, Lacerda Heluy S, Figueiredo LC, Faveri M, Feres M. 2016. The current weight of evidence of the microbiologic profile associated with peri-implantitis: A systematic review. Journal of periodontology. 87(11):1295-1304.

Persson GR, Renvert S. 2014. Cluster of bacteria associated with peri-implantitis. Clinical implant dentistry and related research. 16(6):783-793.

Persson GR, Samuelsson E, Lindahl C, Renvert S. 2010. Mechanical non-surgical treatment of peri-implantitis: A single-blinded randomized longitudinal clinical study. Ii. Microbiological results. Journal of clinical periodontology. 37(6):563-573.

Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. 2005. The global burden of oral diseases and risks to oral health. Bulletin of the World Health Organization. 83:661-669.

Poirier P, Alpert MA, Fleisher LA, Thompson PD, Sugerman HJ, Burke LE, Marceau P, Franklin BA. 2009. Cardiovascular evaluation and management of severely obese patients undergoing surgery: A science advisory from the american heart association. Circulation. 120(1):86-95.

Pothineni NVK, Subramany S, Kuriakose K, Shirazi LF, Romeo F, Shah PK, Mehta JL. 2017. Infections, atherosclerosis, and coronary heart disease. European Heart Journal. 38(43):3195-3201.

Preshaw PM, Heasman PA. 2002. Prostaglandin e2 concentrations in gingival crevicular fluid: Observations in untreated chronic periodontitis. J Clin Periodontol. 29(1):15-20.

Pussinen PJ, Alfthan G, Tuomilehto J, Asikainen S, Jousilahti P. 2004. High serum antibody levels to porphyromonas gingivalis predict myocardial infarction. European Journal of Cardiovascular Prevention & Rehabilitation. 11(5):408-411.

Pyysalo MJ, Pyysalo LM, Pessi T, Karhunen PJ, Öhman JE. 2013. The connection between ruptured cerebral aneurysms and odontogenic bacteria. Journal of Neurology, Neurosurgery & Psychiatry. 84(11):1214-1218.

Quist-Paulsen P. 2010. Statins and inflammation: An update. Curr Opin Cardiol. 25(4):399-405.

Rafferty B, Jönsson D, Kalachikov S, Demmer RT, Nowygrod R, Elkind MS, Bush H, Jr., Kozarov E. 2011. Impact of monocytic cells on recovery of uncultivable bacteria from atherosclerotic lesions. J Intern Med. 270(3):273-280.

Rakic M, Grusovin MG, Canullo L. 2016. The microbiologic profile associated with peri-implantitis in humans: A systematic review. The International journal of oral & maxillofacial implants. 31(2):359-368.

Rakic M, Lekovic V, Nikolic-Jakoba N, Vojvodic D, Petkovic-Curcin A, Sanz M. 2013. Bone loss biomarkers associated with peri-implantitis. A cross-sectional study. Clin Oral Implants Res. 24(10):1110-1116.

Rakic M, Struillou X, Petkovic-Curcin A, Matic S, Canullo L, Sanz M, Vojvodic D. 2014. Estimation of bone loss biomarkers as a diagnostic tool for peri-implantitis. Journal of periodontology. 85(11):1566-1574.

Recker EN, Avila-Ortiz G, Fischer CL, Pagan-Rivera K, Brogden KA, Dawson DV, Elangovan S. 2015. A cross-sectional assessment of biomarker levels around implants versus natural teeth in periodontal maintenance patients. Journal of periodontology. 86(2):264-272.

Reichert S, Schulz S, Benten AC, Lutze A, Seifert T, Schlitt M, Werdan K, Hofmann B, Wienke A, Schaller HG. 2016. Periodontal conditions and incidence of new cardiovascular events among patients with coronary vascular disease. Journal of clinical periodontology. 43(11):918-925.

Renvert S, Aghazadeh A, Hallström H, Persson GR. 2014. Factors related to peri-implantitis–a retrospective study. Clinical oral implants research. 25(4):522-529.

Renvert S, Lindahl C, Renvert H, Persson GR. 2008. Clinical and microbiological analysis of subjects treated with brånemark or astratech implants: A 7-year follow-up study. Clin Oral Implants Res. 19(4):342-347.

Renvert S, Ohlsson O, Pettersson T, Persson GR. 2010. Periodontitis: A future risk of acute coronary syndrome? A follow-up study over 3 years. Journal of periodontology. 81(7):992-1000.

Renvert S, Persson GR, Pirih FQ, Camargo PM. 2018. Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. Journal of periodontology. 89(S1):S304-S312.

Renvert S, Roos-Jansåker AM, Lindahl C, Renvert H, Rutger Persson G. 2007. Infection at titanium implants with or without a clinical diagnosis of inflammation. Clinical oral implants research. 18(4):509-516.

Renvert S, Widén C, Persson RG. 2017. Cytokine and microbial profiles in relation to the clinical outcome following treatment of peri-implantitis. Clin Oral Implants Res. 28(9):1127-1132.

Reyes L, Herrera D, Kozarov E, Roldán S, Progulske-Fox A. 2013. Periodontal bacterial invasion and infection: Contribution to atherosclerotic pathology. J Clin Periodontol. 40 Suppl 14:S30-50.

Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. 2000. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation. 101(15):1767-1772.

Rosen P, Clem D, Cochran D, Froum S, McAllister B, Renvert S, Wang HL. 2013. Peri-implant mucositis and peri-implantitis: A current understanding of their diagnoses and clinical implications. Journal of periodontology. 84(4):436-443.
Roth GA, Forouzanfar MH, Moran AE, Barber R, Nguyen G, Feigin VL, Naghavi M, Mensah GA, Murray CJ. 2015. Demographic and epidemiologic drivers of global cardiovascular mortality. N Engl J Med. 372(14):1333-1341.
Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, Ahmed M, Aksut B, Alam T, Alam K et al. 2017. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. J Am Coll Cardiol. 70(1):1-25.
Ryan ME, Golub LM. 2000. Modulation of matrix metalloproteinase activities in periodontitis as a treatment strategy. Periodontol 2000. 24:226-238.
Rydén L, Buhlin K, Ekstrand E, de Faire U, Gustafsson A, Holmer J, Kjellström B, Lindahl B, Norhammar A, Nygren Å. 2016. Periodontitis increases the risk of a first myocardial infarction: A report from the parokrank study. Circulation. 133(6):576-583.
Sahrmann P, Gilli F, Wiedemeier DB, Attin T, Schmidlin PR, Karygianni L. 2020. The microbiome of peri-implantitis: A systematic review and meta-analysis. Microorganisms. 8(5).
Salgado-Pabón W, Breshears L, Spaulding AR, Merriman JA, Stach CS, Horswill AR, Peterson ML, Schlievert PM. 2013. Superantigens are critical for staphylococcus aureus infective endocarditis, sepsis, and acute kidney injury. mBio. 4(4):e00494-00413.
Salvi GE, Aglietta M, Eick S, Sculean A, Lang NP, Ramseier CA. 2012. Reversibility of experimental peri-implant mucositis compared with experimental gingivitis in humans. Clinical oral implants research. 23(2):182-190.
Salvi GE, Fürst MM, Lang NP, Persson GR. 2008. One-year bacterial colonization patterns of staphylococcus aureus and other bacteria at implants and adjacent teeth. Clin Oral Implants Res. 19(3):242-248.
Sanz M, Alandez J, Lazaro P, Calvo J, Quirynen M, van Steenberghe D. 1991. Histo-pathologic characteristics of peri-implant soft tissues in brånemark implants with 2 distinct clinical and radiological patterns. Clinical oral implants research. 2(3):128-134.
Sanz M, Ceriello A, Buysschaert M, Chapple I, Demmer RT, Graziani F, Herrera D, Jepsen S, Lione L, Madianos P et al. 2018. Scientific evidence on the links between periodontal diseases and diabetes: Consensus report and guidelines of the joint workshop on periodontal diseases and diabetes by the international diabetes federation and the european federation of periodontology. J Clin Periodontol. 45(2):138-149.
Sanz M, Chapple IL, Periodontology* WGotVEWo. 2012. Clinical research on peri-implant diseases: Consensus report of w orking g roup 4. Journal of clinical periodontology. 39:202-206.
Sanz M, Marco del Castillo A, Jepsen S, Gonzalez-Juanatey JR, D'Aiuto F, Bouchard P, Chapple I, Dietrich T, Gotsman I, Graziani F et al. 2020. Periodontitis and cardiovascular diseases: Consensus report. Journal of Clinical Periodontology. 47(3):268-288.
Sattar N, McCarey DW, Capell H, McInnes IB. 2003. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation. 108(24):2957-2963.
Schenkein HA, Loos BG. 2013. Inflammatory mechanisms linking periodontal diseases to cardiovascular diseases. J Clin Periodontol. 40 Suppl 14(0 14):S51-69.
Schenkein HA, Papapanou PN, Genco R, Sanz M. 2020. Mechanisms underlying the association between periodontitis and atherosclerotic disease. Periodontol 2000. 83(1):90-106.
Schimmel M, Srinivasan M, McKenna G, Müller F. 2018. Effect of advanced age and/or systemic medical conditions on dental implant survival: A systematic review and meta-analysis. Clinical oral implants research. 29:311-330.
Schoppet M, Al-Fakhri N, Franke FE, Katz N, Barth PJ, Maisch B, Preissner KT, Hofbauer LC. 2004. Localization of osteoprotegerin, tumor necrosis factor-related apoptosis-inducing ligand, and receptor activator of nuclear factor-kappab ligand in mönckeberg's sclerosis and atherosclerosis. J Clin Endocrinol Metab. 89(8):4104-4112.
Schoppet M, Preissner KT, Hofbauer LC. 2002. Rank ligand and osteoprotegerin: Paracrine regulators of bone metabolism and vascular function. Arterioscler Thromb Vasc Biol. 22(4):549-553.
Schwarz F, Becker K, Rahn S, Hegewald A, Pfeffer K, Henrich B. 2015. Real-time pcr analysis of fungal organisms and bacterial species at peri-implantitis sites. Int J Implant Dent. 1(1):9.
Schwarz F, Derks J, Monje A, Wang H-L. 2018. Peri-implantitis. Journal of periodontology. 89(S1):S267-S290.
Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12(6):R60.
Sen S, Giamberardino LD, Moss K, Morelli T, Rosamond WD, Gottesman RF, Beck J, Offenbacher S. 2018. Periodontal disease, regular dental care use, and incident ischemic stroke. Stroke. 49(2):355-362.

Shaker OG, El-Shehaby A, Nabih M. 2010. Possible role of osteoprotegerin and tumor necrosis factor-related apoptosis-inducing ligand as markers of plaque instability in coronary artery disease. Angiology. 61(8):756-762.
Sharma P, Dietrich T, Ferro CJ, Cockwell P, Chapple IL. 2016. Association between periodontitis and mortality in stages 3–5 chronic kidney disease: Nhanes iii and linked mortality study. Journal of clinical periodontology. 43(2):104-113.
Shibli JA, Melo L, Ferrari DS, Figueiredo LC, Faveri M, Feres M. 2008. Composition of supra- and subgingival biofilm of subjects with healthy and diseased implants. Clin Oral Implants Res. 19(10):975-982.
Shin D, Kongpakpaisarn K, Bohra C. 2018. Trends in the prevalence of metabolic syndrome and its components in the united states 2007–2014. International journal of cardiology. 259:216-219.
Shin D-S, Park J-W, Suh J-Y, Lee J-M. 2010. The expressions of inflammatory factors and tissue inhibitor of matrix metalloproteinase-2 in human chronic periodontitis with type 2 diabetes mellitus. Journal of periodontal & implant science. 40(1):33-38.
Smit J, Adelborg K, Thomsen RW, Søgaard M, Schønheyder HC. 2016. Chronic heart failure and mortality in patients with community-acquired staphylococcus aureus bacteremia: A population-based cohort study. BMC Infect Dis. 16:227-227.
Sohail MR, Palraj BR, Khalid S, Uslan DZ, Al-Saffar F, Friedman PA, Hayes DL, Lohse CM, Wilson WR, Steckelberg JM et al. 2015. Predicting risk of endovascular device infection in patients with staphylococcus aureus bacteremia (predict-sab). Circ Arrhythm Electrophysiol. 8(1):137-144.
Sorsa T, Bacigalupo J, Könönen M, Pärnänen P, Räisänen IT. 2020. Host-modulation therapy and chair-side diagnostics in the treatment of peri-implantitis. Biosensors (Basel). 10(5):44.
Sorsa T, Gursoy UK, Nwhator S, Hernandez M, Tervahartiala T, Leppilahti J, Gursoy M, Könönen E, Emingil G, Pussinen PJ et al. 2016. Analysis of matrix metalloproteinases, especially mmp-8, in gingival creviclular fluid, mouthrinse and saliva for monitoring periodontal diseases. Periodontol 2000. 70(1):142-163.
Stefan N, Häring H-U, Hu FB, Schulze MB. 2013. Metabolically healthy obesity: Epidemiology, mechanisms, and clinical implications. The lancet Diabetes & endocrinology. 1(2):152-162.
Stetler-Stevenson WG. 2008. The tumor microenvironment: Regulation by mmp-independent effects of tissue inhibitor of metalloproteinases-2. Cancer Metastasis Rev. 27(1):57-66.
Teeuw WJ, Slot DE, Susanto H, Gerdes VE, Abbas F, D'Aiuto F, Kastelein JJ, Loos BG. 2014. Treatment of periodontitis improves the atherosclerotic profile: A systematic review and meta-analysis. J Clin Periodontol. 41(1):70-79.
Teronen O, Konttinen YT, Lindqvist C, Salo T, Ingman T, Lauhio A, Ding Y, Santavirta S, Sorsa T. 1997. Human neutrophil collagenase mmp-8 in peri-implant sulcus fluid and its inhibition by clodronate. J Dent Res. 76(9):1529-1537.
Thapa S, Wei F. 2016. Association between high serum total cholesterol and periodontitis: National health and nutrition examination survey 2011 to 2012 study of american adults. Journal of periodontology. 87(11):1286-1294.
Tomás I, Diz P, Tobías A, Scully C, Donos N. 2012. Periodontal health status and bacteraemia from daily oral activities: Systematic review/meta-analysis. Journal of Clinical Periodontology. 39(3):213-228.
Tonetti M, Van Dyke T. 2013. Efpaapw working group 1 of the joint. Periodontitis and atherosclerotic cardiovascular disease: Consensus report of the joint efp/aap workshop on periodontitis and systemic diseases. J Clin Periodontol. 40.
Tonetti MS, Greenwell H, Kornman KS. 2018. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. Journal of periodontology. 89(S1):S159-S172.
Tsai C, Hayes C, Taylor GW. 2002. Glycemic control of type 2 diabetes and severe periodontal disease in the us adult population. Community dentistry and oral epidemiology. 30(3):182-192.
Tuck MK, Chan DW, Chia D, Godwin AK, Grizzle WE, Krueger KE, Rom W, Sanda M, Sorbara L, Stass S et al. 2009. Standard operating procedures for serum and plasma collection: Early detection research network consensus statement standard operating procedure integration working group. J Proteome Res. 8(1):113-117.
Umare V, Pradhan V, Nadkar M, Rajadhyaksha A, Patwardhan M, Ghosh KK, Nadkarni AH. 2014. Effect of proinflammatory cytokines (il-6, tnf-α, and il-1β) on clinical manifestations in indian sle patients. Mediators of Inflammation. 2014:385297.
Ungprasert P, Koster MJ, Warrington KJ, Matteson EL. 2017. Polymyalgia rheumatica and risk of coronary artery disease: A systematic review and meta-analysis of observational studies. Rheumatol Int. 37(1):143-149.
Velsko IM, Chukkapalli SS, Rivera MF, Lee JY, Chen H, Zheng D, Bhattacharyya I, Gangula PR, Lucas AR, Kesavalu L. 2014. Active invasion of oral and aortic tissues by porphyromonas gingivalis in mice causally links periodontitis and atherosclerosis. PLoS One. 9(5):e97811.

Velsko IM, Chukkapalli SS, Rivera-Kweh MF, Chen H, Zheng D, Bhattacharyya I, Gangula PR, Lucas AR, Kesavalu L. 2015. Fusobacterium nucleatum alters atherosclerosis risk factors and enhances inflammatory markers with an atheroprotective immune response in apoe(null) mice. PloS one. 10(6):e0129795-e0129795.

Venza I, Visalli M, Cucinotta M, De Grazia G, Teti D, Venza M. 2010. Proinflammatory gene expression at chronic periodontitis and peri-implantitis sites in patients with or without type 2 diabetes. Journal of periodontology. 81(1):99-108.

Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Delling FN. 2020. Heart disease and stroke statistics—2020 update: A report from the american heart association. Circulation.E139-E596.

Wang HL, Garaicoa-Pazmino C, Collins A, Ong HS, Chudri R, Giannobile WV. 2016. Protein biomarkers and microbial profiles in peri-implantitis. Clin Oral Implants Res. 27(9):1129-1136.

Wensley F, Gao P, Burgess S, Kaptoge S, Di Angelantonio E, Shah T, Engert J, Clarke R, Davey-Smith G, Nordestgaard B. 2011. C reactive protein coronary heart disease genetics collaboration (ccgc). Association between c reactive protein and coronary heart disease: Mendelian randomisation analysis based on individual participant data. Bmj. 342(2):548-556.

Whelton PK, Carey RM, Aronow WS, Casey DE, Collins KJ, Himmelfarb CD, DePalma SM, Gidding S, Jamerson KA, Jones DW et al. 2018. 2017 acc/aha/aapa/abc/acpm/ags/apha/ash/aspc/nma/pcna guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: A report of the american college of cardiology/american heart association task force on clinical practice guidelines. Hypertension. 71(6):e13-e115.

Wood AM, Kaptoge S, Butterworth AS, Willeit P, Warnakula S, Bolton T, Paige E, Paul DS, Sweeting M, Burgess S et al. 2018. Risk thresholds for alcohol consumption: Combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. Lancet. 391(10129):1513-1523.

Obesity: Preventing and managing the global epidemic. Report of a who consultation on obesity. . 1997. [accessed 2002]. http://dosei.who.int/uhtbin/cgisirsi/Wed+Nov+21+23:21:20+MET+2001/0/49.

World Health Organization. 2014. Global status report on noncommunicable diseases 2014 (updated in 2018). World Health Organization.

Xu L, Yu Z, Lee HM, Wolff MS, Golub LM, Sorsa T, Kuula H. 2008. Characteristics of collagenase-2 from gingival crevicular fluid and peri-implant sulcular fluid in periodontitis and peri-implantitis patients: Pilot study. Acta Odontol Scand. 66(4):219-224.

Yaghobee S, Khorsand A, Rasouli Ghohroudi AA, Sanjari K, Kadkhodazadeh M. 2014. Assessment of interleukin-1beta and interleukin-6 in the crevicular fluid around healthy implants, implants with peri-implantitis, and healthy teeth: A cross-sectional study. Journal of the Korean Association of Oral and Maxillofacial Surgeons. 40(5):220-224.

Yalçn S, Baseğmez C, Mijiritsky E, Yalçn F, Isik G, Onan U. 2005. Detection of implant crevicular fluid prostaglandin e2 levels for the assessment of peri-implant health: A pilot study. Implant Dent. 14(2):194-200.

Yang J, Wu J, Liu Y, Huang J, Lu Z, Xie L, Sun W, Ji Y. 2014. Porphyromonas gingivalis infection reduces regulatory t cells in infected atherosclerosis patients. PLoS One. 9(1):e86599.

Yang S, Zhao LS, Cai C, Shi Q, Wen N, Xu J. 2018. Association between periodontitis and peripheral artery disease: A systematic review and meta-analysis. BMC cardiovascular disorders. 18(1):141.

Yong WC, Sanguankeo A, Upala S. 2018. Association between primary sjögren's syndrome, cardiovascular and cerebrovascular disease: A systematic review and meta-analysis. Clin Exp Rheumatol. 36 Suppl 112(3):190-197.

Yousuf O, Mohanty BD, Martin SS, Joshi PH, Blaha MJ, Nasir K, Blumenthal RS, Budoff MJ. 2013. High-sensitivity c-reactive protein and cardiovascular disease: A resolute belief or an elusive link? Journal of the American College of Cardiology. 62(5):397-408.

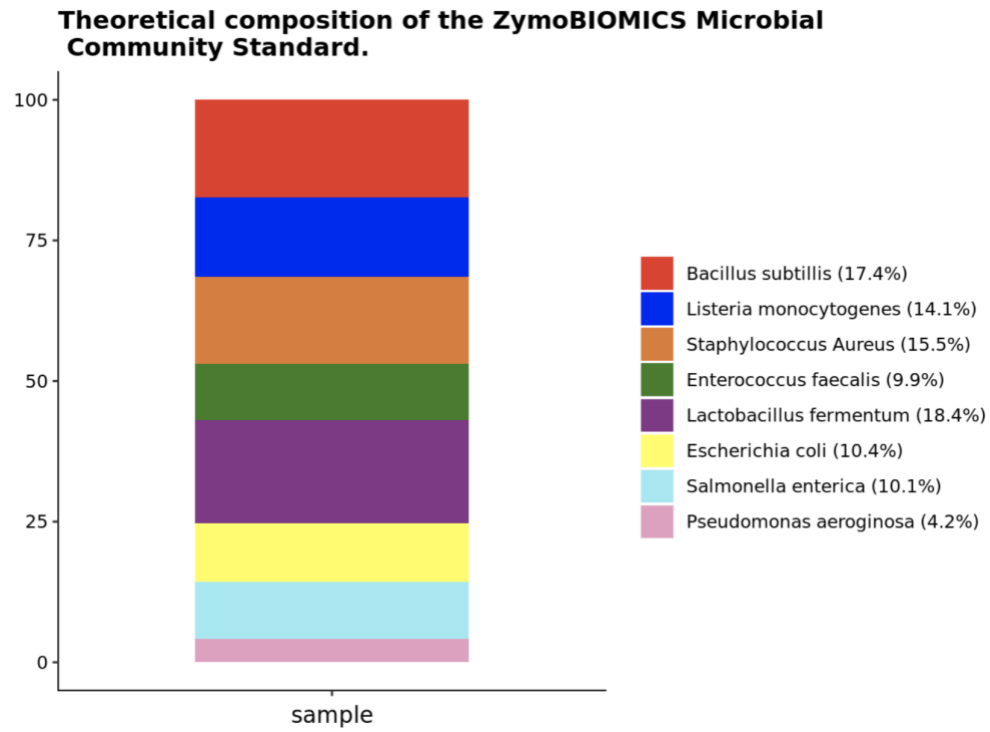
Yu YH, Chasman DI, Buring JE, Rose L, Ridker PM. 2015. Cardiovascular risks associated with incident and prevalent periodontal disease. Journal of clinical periodontology. 42(1):21-28.

Zhuang LF, Watt RM, Mattheos N, Si MS, Lai HC, Lang NP. 2016. Periodontal and peri-implant microbiota in patients with healthy and inflamed periodontal and peri-implant tissues. Clin Oral Implants Res. 27(1):13-21.

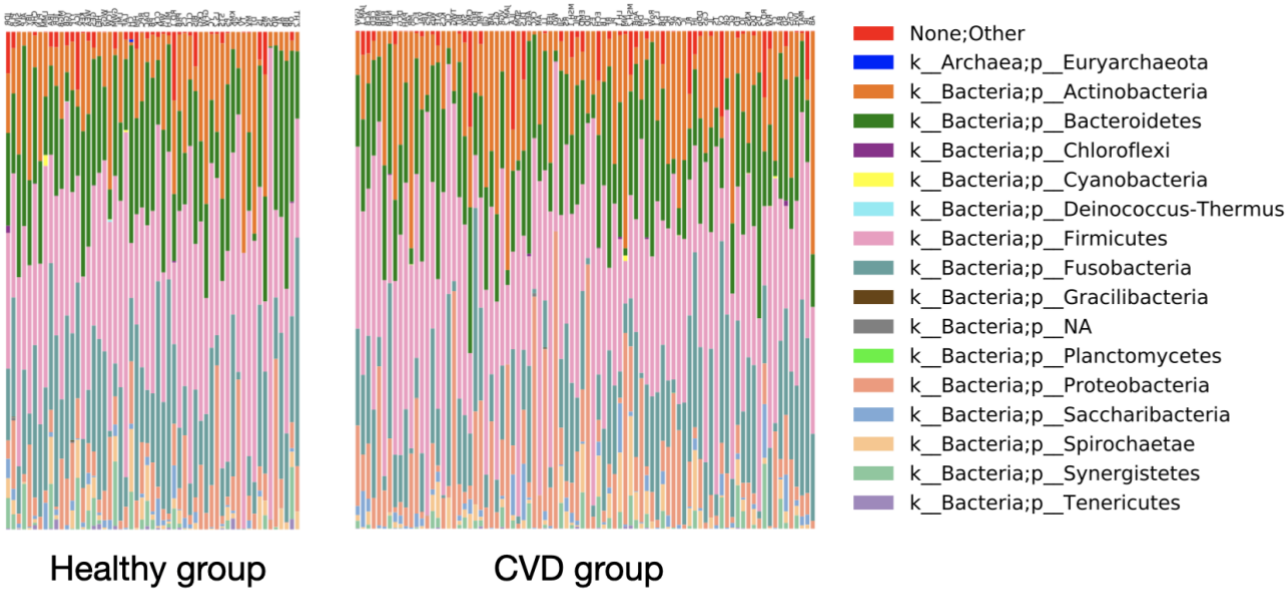
Zitzmann N, Berglundh T, Marinello C, Lindhe J. 2001. Experimental peri-implant mucositis in man. Journal of clinical periodontology. 28(6):517-523.

Chapter IX. Supplemental Tables and Figures

Supplemental Figure 1. Theoretical microbial composition of the ZymoBIOMICS® Microbial Community Standard (positive control)



Supplemental Figure 2. Comparison between non-CVD and CVD group at the Phylum level
Supplemental Figure 3. Comparison between non-CVD and CVD group at the Class level



★ denotes the periodontitis-associated bacterial classes; red font indicated the percentage in the microbial community of individual Class among CVD group compared to the black font (healthy group)

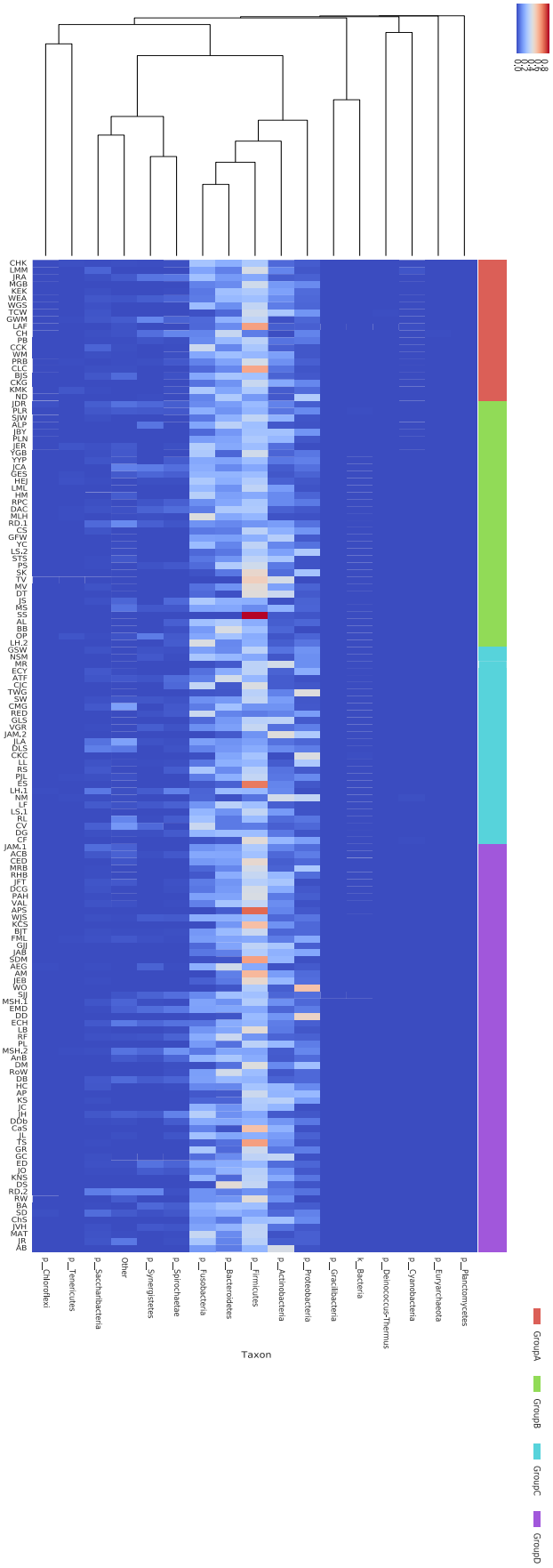
Supplemental Table 1. Composition of Taxonomy at the Class level

	non-CVD	CVD
k__bacterial;p__Firmicutes;c__Bacilli	17.42%	19.34%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia	17.40%	16.30%
k__Bacteria;p__Fusobacteria;c__Fusobacteriia	18.47%	15.18%
k__Bacteria;p__Actinobacteria;c__Actinobacteria	10.78%	12.66%
k__Bacteria;p__Firmicutes;c__Clostridia	10.65%	8.82%
k__Bacteria;p__Firmicutes;c__Negativicutes	6.12%	6.24%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria	3.09%	4.11%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria	1.07%	3.78%
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria	2.66%	2.55%
None;Other;Other	1.80%	2.25%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia	2.14%	2.01%
k__Bacteria;p__Spirochaetae;c__Spirochaetes	1.93%	1.92%
k__Bacteria;p__Saccharibacteria;c__NA	1.03%	1.42%
k__Bacteria;p__Synergistetes;c__Synergistia	1.81%	1.32%

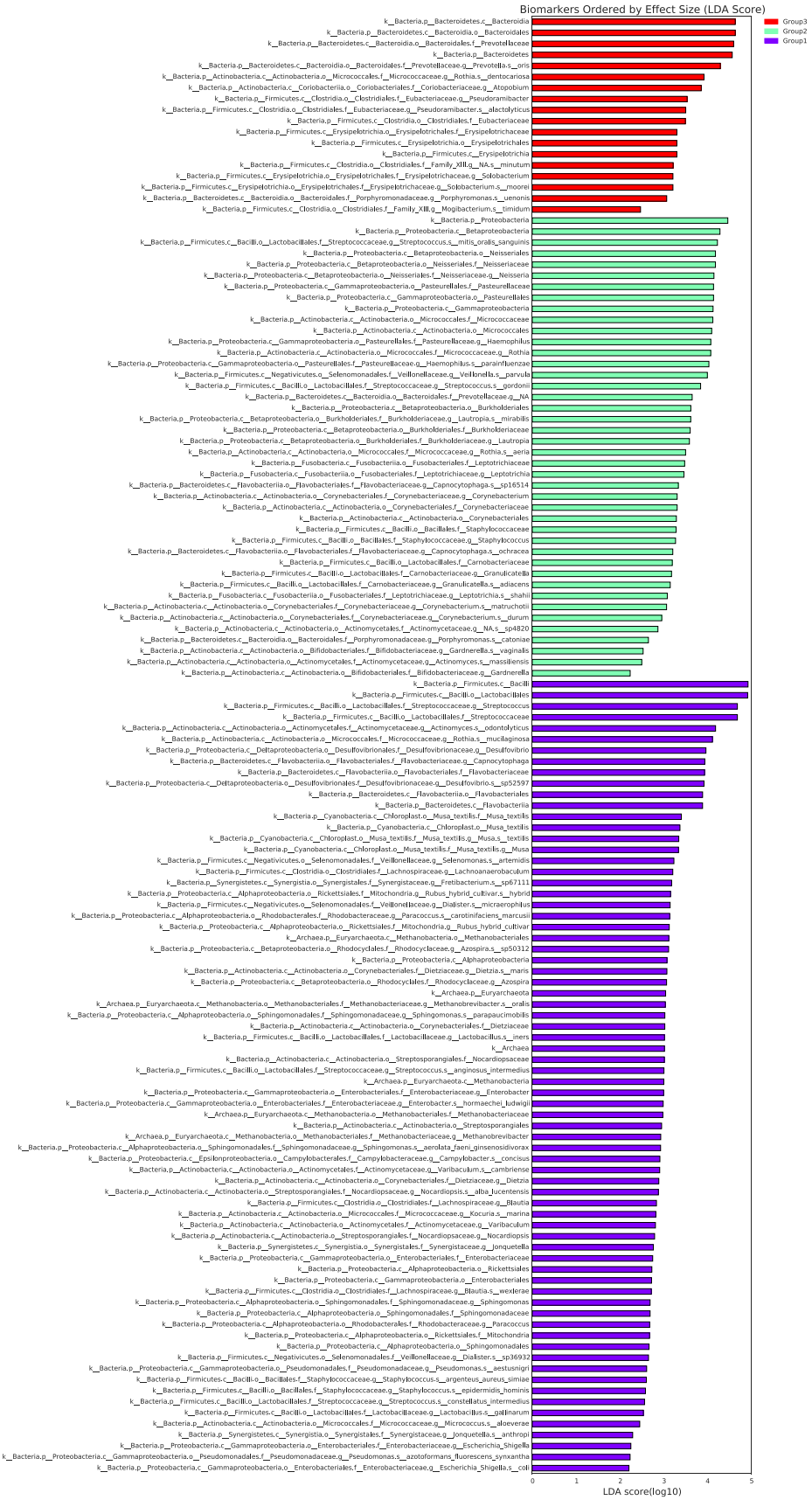
*Font in bold indicated the classes associated with periodontitis

Supplemental Figure 4. Heatmap of 4 groups comparison on phylum level

Group A= non-CVD + healthy implant
Group B= non-CVD + peri-implantitis
Group C= CVD + healthy implant
Group D= CVD + per-implantitis)



Supplemental Figure 5. LEfSe analysis of different peri-implant pocket depths

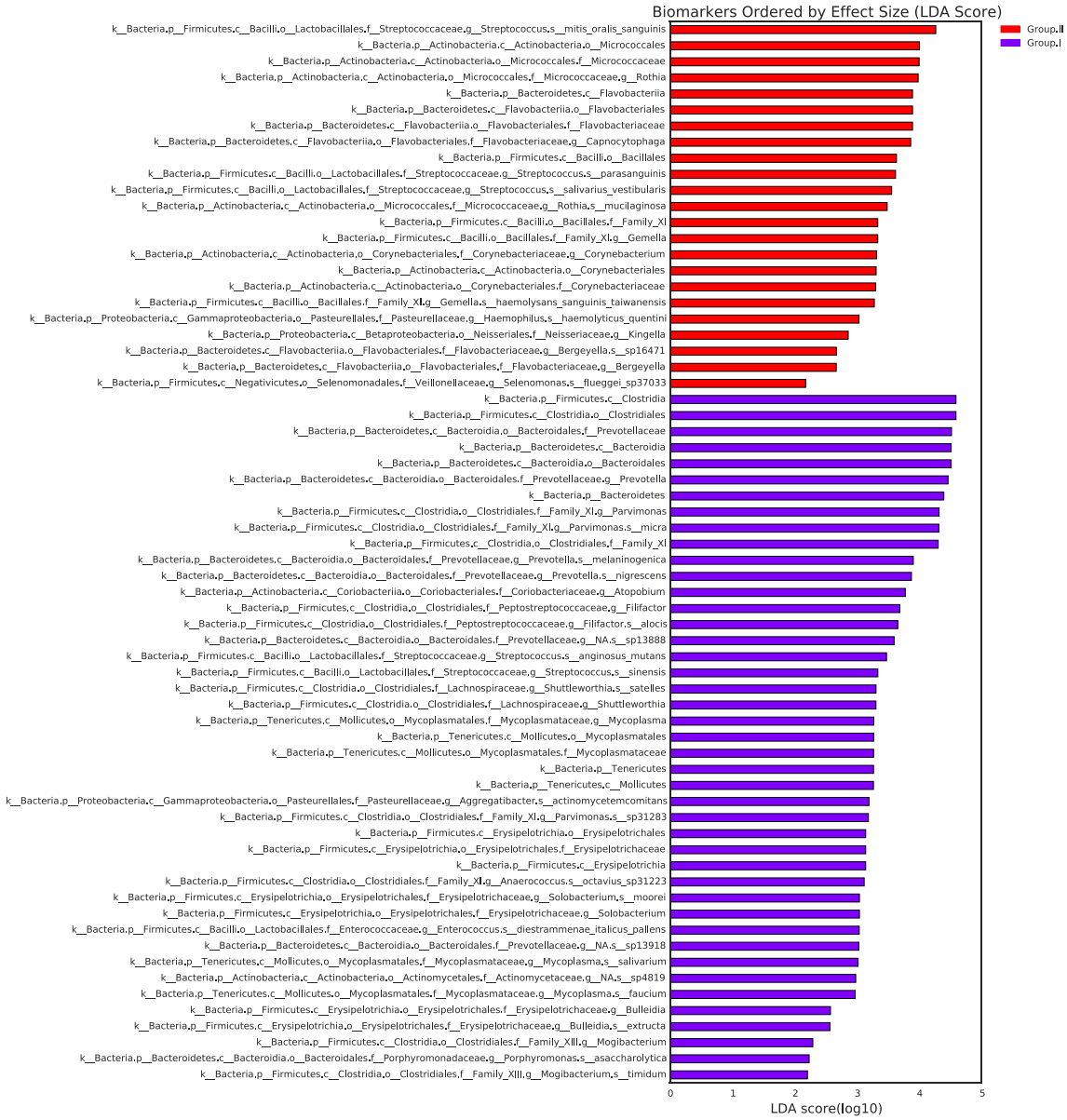


Group 1 (*purple*): PPD ≤3mm

Group 2 (*green*): PPD 4-6 mm

Group 3 (*red*): ≥ 7 mm

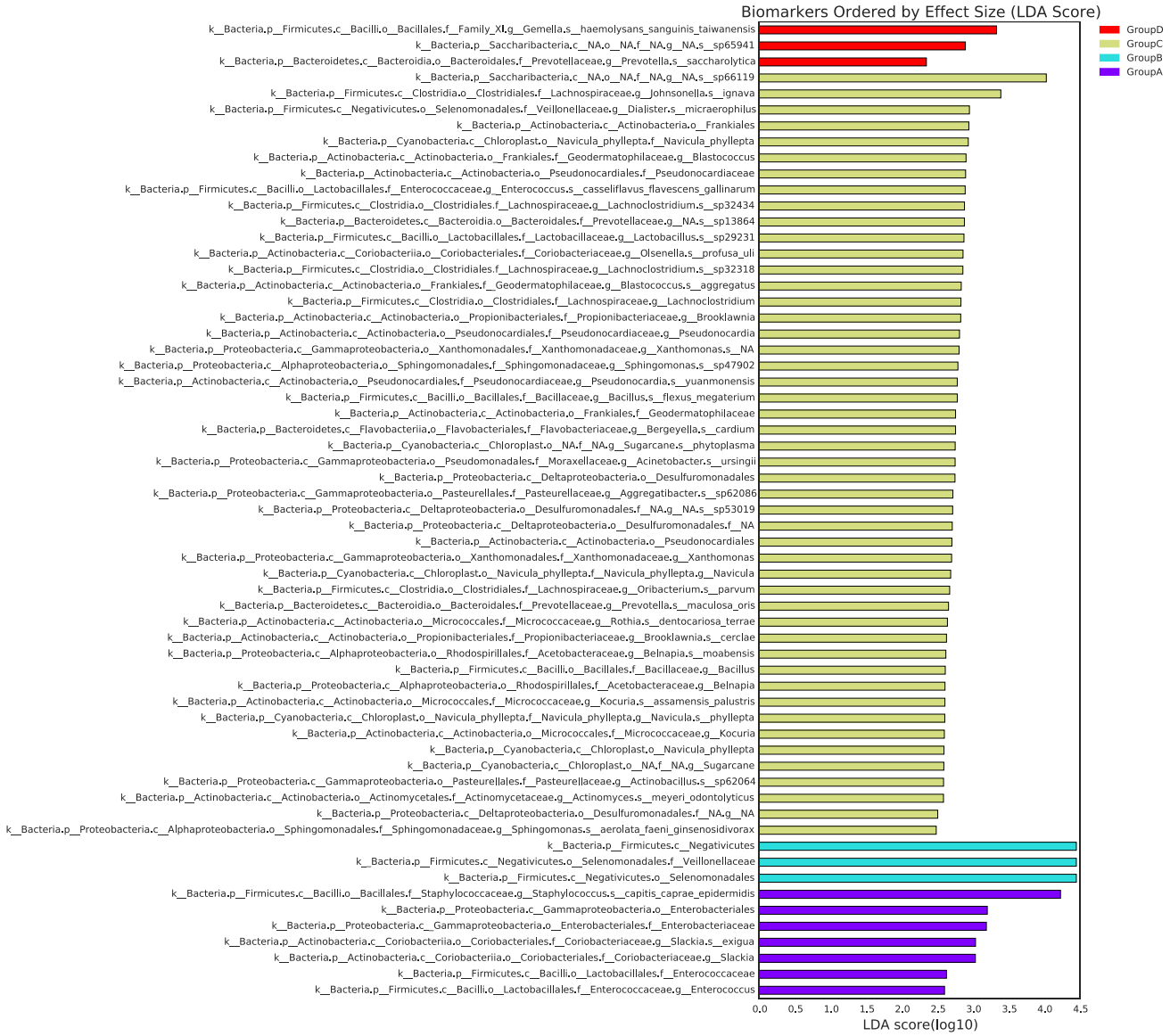
Supplemental Figure 6. LEfSe analysis of peri-implant pockets with or without suppuration



Group I (*purple*): presence of suppuration

Group II (*red*): absence of suppuration

Supplemental Figure 7. LEfSe analysis of pockets with peri-implantitis with simultaneous periodontitis



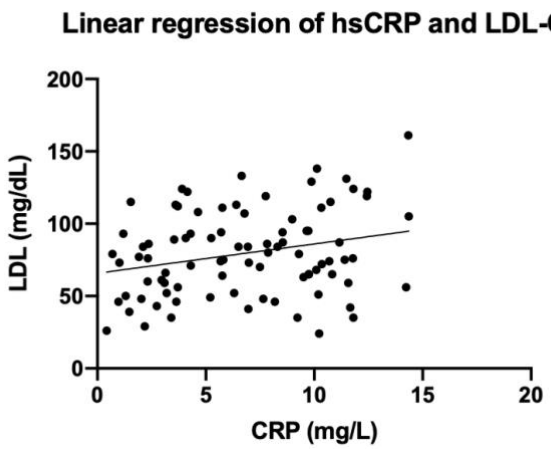
Group A (*purple*): Peri-implantitis +Stage 1 periodontitis
Group B (*green*): Peri-implantitis +Stage 2 periodontitis
Group C (*yellow*): Peri-implantitis +Stage 3 periodontitis
Group D (*red)* : Peri-implantitis +Stage 4 periodontitis

Supplemental Table 2. Discussion of findings of serum hsCRP

Serum hsCRP

Serum hsCRP, a downstream marker for inflammation, has been considered as an important predictor for CVD risk. It was categorized as low risk (hsCRP<1 mg/L), intermediate risk (1-3 mg/L) and high risk (>3 mg/L) by (Greenland et al. 2010)). Serum hsCRP levels over 10 mg/L is correlated with an over 4% global risk of developing a fatal CVD in 10 years (Cozlea et al. 2013). hsCRP is produced in the liver in response to infection or inflammatory disease, such as RA, systemic lupus erythematous (SLE), or cancer. Phenotypes that predispose accelerated atherosclerosis including metabolic syndrome, visceral obesity, and insulin resistance were significantly associated with elevated levels of hsCRP (Yousuf et al. 2013). Adipose tissue, a significant source of IL-6, may explain the robust association between hsCRP and obesity (Buckley et al. 2009). It has been widely accepted that low grade systemic inflammation, and consequently an elevated hsCRP, contributes to an elevated risk of CVD (Teeuw et al. 2014). A meta-analysis of 54 long-term prospective studies reported the CRP concentration was linearly associated with several risk factors and inflammatory markers, and nearly log-linearly with the risk of ischemic vascular disease and non-vascular mortality (Kaptoge et al. 2010). However, its predictive value has been proved to be limited. First, a lack of a causative relationship between CRP and CHD has been suggested by animal and human genetic data (Elliott et al. 2009; Koike et al. 2009; Dehghan et al. 2011). A meta-analysis of 46,557 patients with CHD and 147,861 controls confirmed the null association among CRP-related genotypes, traditional risk, and risk of CHD (Wensley et al. 2011). Secondly, the variability among individuals (genetic polymorphisms, sex, ethic, BMI, hypertension, periodontal disease, environmental pollutant burden, and lifestyle) and technical sensitivity could contribute to the massive heterogeneity. In the current investigation, CRP was not associated with CVD occurrence, even among those with LDL-C < 130 mg/dl, for whom the use of hsCRP has been advocated by some (Detrano et al. 2008; Blankstein et al. 2011). Additional analysis showed that it was positively correlated with the LDL-C level in the linear regression model (Slope: 2.0, p=0.03, R-square= 0.07) (Supplemental Figure 8). It is understandable since CRP directly bonds to atherogenic oxidized LDL cholesterol and is present within lipid-laden plaques (Libby et al. 2010). The current result that average levels of hsCRP was higher in the non-CVD group may be correlated to the higher percentage of participants with abnormal LDL-C.

Supplemental Figure 8. Linear relationship between LDL cholesterol and serum CRP



Supplemental Table 3. Discussion of findings of PICF non-significant biomarkers of OPG, IL-6, IL-8, VEGF

PICF non-significant biomarkers
Although only a trend of higher OPG and IL-6 at peri-implant diseased sites was noted, a notably increased concentration isolated from the GCF among patients with periodontitis was found in the current investigation. A significantly higher peri-implant concentration of bone remodeling biomarkers (RANK, OPG, and RANKL) has been reported in peri-implantitis sites(Duarte et al. 2009a; Rakic et al. 2014). RANKL and RANKL/OPG ratio have been documented to be higher in the periodontitis pockets as well (Rakic et al. 2013). It has been reported that a significantly higher level of pro-inflammatory cytokine IL-6 is found at peri-implantitis sites (Yaghobee et al. 2014). It is in agreement with a meta-analysis that IL-6 is found higher in sites with peri-implantitis than in peri-implant mucositis sites (Ghassib et al. 2019). However, some had also reported that IL-1β and IL-6 are not significantly different between healthy implants and implants with peri-implantitis (Melo et al. 2012). Our investigation supported a link between

elevated IL-8 in sites with peri-implantitis and periodontitis, which was in line with the previous findings(Venza et al. 2010). Similar studies, which have shown IL-8 secretion is highly correlated with IL-1 secretion (Payne et al. 1993) as well as the clinical manifestation of periodontal diseases (Tsai et al. 2002; Giannopoulou et al. 2003) can also support our results. Future studies are needed to clearly define the relationship. Lastly, VEGF was found to be higher in peri-implant mucositis than peri-implantitis in the present study, which may be related to the erythma of soft connective tissue affected by the mucositis or infection. VEGF increases vascular permeability and extravasation of plasma proteins to facilitate the migration and oxidation of the augmented inflammatory cells. It has been reported to be lower in peri-implantitis samples, and positively correlated to the inflammatory infiltrates (Cornelini et al. 2001).