Chapter 10

Periodontal Therapy and Cardiovascular Risk

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ABSTRACT

Cardiovascular diseases (CVD) are the worldwide leading cause of mortality. CVD are non-communicable conditions with a complex pathogenesis and their clinical manifestations include major cardiovascular events such as myocardial infarction (MI) and stroke.

Epidemiological evidence suggests a consistent association between Periodontitis and increased risk of CVD. Some evidence supports a beneficial effect of the treatment of periodontitis (PT) on both surrogate and hard cardiovascular (CV) outcomes.

This narrative review has been conducted as an update of the most recent evidence on the effects of PT on CV outcomes..

Newer evidence originating from published randomized controlled trials (RCTs) confirms a positive effect of PT on surrogate measures of CVD whilst there have been no RCTs investigating the effect of PT on the incidence of CVD events such as MI and stroke.

In conclusion, there is sufficient evidence from observational and experimental studies on surrogate CV measures to justify the design and conduct of appropriately powered RCTs investigating the effect of effective periodontal interventions on CVD outcomes (i.e. MI and stroke) with adequate control of CV traditional risk factors.

Introduction

Periodontitis (PD) is a chronic infectious disease targeting the connective tissue and alveolar bone supporting the dentition[1]. A progressive deterioration of periodontal health is linked to the accumulation of specific oral microorganisms able to trigger tissue damage and also affect the balance of the different species present in the dental biofilm switching from a symbiotic into a dysbiotic state by altering the normal homeostatic relationship with the host[2]. The inflammatory response to these pathogens is responsible for the destruction of the periodontium[3].

According to its extent and severity, and if left untreated for years, PD is a leading cause of tooth loss. The chronic infectious and inflammatory nature of this disorder pose the question of whether it might also have a systemic reflection. Alternating periods of interest and dismissal of a possible causal relationship between oral and systemic health have been reported since the concept of "focal infection" was proposed.

In 1785 a relationship between infection of the tonsils and some systemic diseases was suggested[4]. In 1801, empirical evidence suggested a benefit to arthritis of the hip following a tooth extraction[5]. Ever since great interest in the role of oral infections on systemic health outcomes have sparkled research investigations which too often have proven to be unconvincing and lacking robust results[6].

Several observational studies have explored the potential association between cardiovascular diseases (CVDs) and infections, mainly correlating the antibody titers against a specific pathogen and rate of cardiovascular events suggesting an association between several pathogens and CVD. Cytomegalovirus (CMV)[7], Herpes Simplex Virus (HSV)[8], hepatitis A virus (HAV)[9], human immunodeficiency virus (HIV)[10], human papilloma virus (HPV)[11], Chlamydia Pneumoniae (Cp)[12], Helicobacter Pylori (HP)[13] have been linked to CVD. Epidemiological evidence suggested an increased risk of cardiovascular (CV) events such as myocardial infarction (MI) and stroke during the

acute phase of common infection and a beneficial effect of influenza vaccination in patients with higher CV risk. This lead the American Heart Association/American College of Cardiology (AHA/ACC) to recommend the administration of influenza vaccine for the secondary prevention of coronary and other atherosclerotic vascular diseases[14]. It is not clear how this protection occurs, and it has been suggested that protection for heart disease is partly due to the acutely triggered cardiovascular events account for mortality in patients who have flu.

Future studies of the role of vaccination against viruses may shed light on the role of viruses in the association of periodontal disease to cardiovascular disease and other systemic effects of periodontal disease.

Since 1980' observational evidence was published that dental health was significantly worse in patients with acute myocardial infarction than in controls[15], the hypothesis that that chronic infections, such as PD, could be implicated in the pathogenesis of atherosclerosis were pointed out by prospective results of a large population study[16]. Ultimately, the evidence of a strong inflammatory component in the pathogenesis of atherosclerosis[17] have given strength of a plausible mechanism linking PD and CVDs. Indeed numerous clinical trials reported increased systemic inflammatory profiles (acute phase reactants, cytokines) in patients with PD compared to controls and decrease in inflammatory mediators concentrations following periodontal therapy (PT)[18]. Systemic inflammation could therefore, represent the biologic link between PD and CVDs, however the specific mechanisms linking the two are not clear and mechanisms are discussed in Chapter ____.

In 2013 the European Federation of Periodontology (EFP) and the American Academy of Periodontology (AAP) addressed the issue of causality between CVD and PD in a Joint Workshop. A summary of the evidence on the impact of periodontal treatment on traditional markers related to CV

health/risk, surrogate and hard CV outcomes[18] concluded that the data available at that time was supportive of an association between these disorders and it further suggested a beneficial effect of PT on outcomes relative to CVDs. The lack of studies on CV hard outcomes did not allow drawing robust conclusions on the direct effect of PT in reducing the risk of CV events such as myocardial infarction (MI) or stroke.

It is beyond our scope to provide a summary of all the available evidence on this topic for which comprehensive systematic reviews have been published[19]. This narrative review aims to provide a critical analysis of recent studies and current knowledge on the effects of periodontal treatment on CVDs..

PERIODONTAL TREATMENT

Considering the central role of bacteria in the pathogenesis of PD, the first aim of the treatment is to remove the etiological factors contributing to the onset and progression of the disease. The mechanical instrumentation of the supra and sub gingival teeth surface with or without the use of antimicrobials aims to disrupt the biofilm triggering the host inflammatory response. The management of behavioral risk factors such as oral hygiene habits and smoking, local risk factors such as occlusal trauma, calculus and other plaque retentive surfaces (ie defective restorations) and systemic diseases such as diabetes together with regular periodontal supportive therapy is then necessary to reduce the risk of PD progression. The addition of periodontal surgery in form of resective or regenerative procedures, in selected suitable cases can contribute to build more long-term favorable periodontal tissues architecture.

PT might also have systemic repercussions. Evidence available on the topic relates to the beneficial effect of PT on the glycemic control in Type II Diabetes (T2DM). The current evidence suggests that PT could reduce Hb1Ac of of 0.27-0.48% after 3 months follow up[20].

CARDIOVASCULAR DISEASES (CVDs)

Of the non-communicable diseases (NCDs), CVDs are leading causes of death with 17.7 million people dying each year from CVDs, an estimated 31% of all deaths worldwide and with 80% of all CVD deaths due to Acute Myocardial Infarction (AMI) and strokes[21]. In order to lower the NCD-related premature mortality by 25% by 2025, the World Health Organization (WHO) in 2013 has introduced the 25×25 Global Action Plan[22].

Identification of individuals at higher or lower risk for cardiovascular events is important to facilitate effective use of resources and interventions to reduce the disease burden. Age, gender, dyslipidemia, hypertension, diabetes mellitus, and smoking are established risk factors for cardiovascular disease.

A Task force composed by the AHA/ACC in 2014 released guidelines on the assessment of cardiovascular risk[23] and published an algorithm to calculate the 10-year risk of heart disease or stroke (http://www.cvriskcalculator.com/). The Work Group adopted elaborated statistical methods to derive and internally validate the Pooled Cohort Equations, which provide sex-and race-specific estimates of the 10-year risk for atherosclerotic cardiovascular disease (ASCVD) for African-American and White men and women 40 to 79 years of age. The variable included in the calculator were: age, gender, race, total cholesterol (mg/dL), HDL cholesterol (mg/dL), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), treatment for high blood pressure, diabetes and smoking.

Several other potential risk markers had been considered for the algorithm but for many there was no additional utility. CV risk factors such as high-sensitivity C-reactive protein (hs-CRP), coronary artery calcium (CAC) score as measure by electron beam compute tomography, lipoprotein (a) level, leukocytes count, fasting blood glucose, periodontal disease, ankle-brachial index (ABI) and carotid

intima-media thickness (cIMT) were analyzed and for the time being their utility in the CV risk reclassification is still unclear.

Emerging CVD risk factors are summarized in Table 1 and fall into numerous overlapping categories. Recent studies provide evidence for periodontal disease as a source of some of the metabolic markers such as fibrinogen, IL-6, and TNFα.

Table 1 Emerging potential risk markers for CVD
Metabolic/ Dietary markers: Triglycerides, impaired glucose tolerance, metabolic syndrome, leptin, adiponectin, trimethylamine-N-oxide (TMAO)
Novel lipids: LP(a), apoA, apoB
Inflammatory markers: Fibrinogen, PV, WCC, hsCRP, IL-6, TNFα, IL-18, sCD40L, MMPs, α oxLDL, Abs
Markers of endothelial activation/damage: oxLDL, sICAM-1, NO, brachial artery reactivity, glutathione dysfunction
Thrombotic markers: t-PA, PAI-1, D-dimer, vWF, homocysteine
Non-invasive imaging biomarkers: Ankle brachial pressure index (ABI), MRI angiography, carotid IMT, CT coronary calcification
Invasive imaging biomarkers: Intravascular ultrasound, coronary angiography

TREATMENT OF CVD

The strategies of primary and secondary prevention of CVD represent a long-term effort of all health care professionals and the basis of many public health policies. The aim of both individual practitioners and organizations providing health care is to reduce the risk factors before clinical

atherosclerotic disease becomes clinically evident.

Lifestyle intervention

The ACC/AHA 2013 Guideline on Lifestyle Management to Reduce Cardiovascular Risk suggests lifestyle interventions evaluated in randomized clinical trial relying primarily on biomarkers or surrogate endpoints rather than "hard" cardiovascular outcomes[24]. The health risks related to tobacco should be provided encouraging smoking cessation. Similarly, prudent dietary and physical activity habits for maintaining ideal body weight should be recommended. Both National Institutes of Health (NIH) and AHA statements recommend at least 30 min of moderate-intensity physical activity per day for 5 days per week. Obesity, specifically the pattern of centripetal or visceral fat accumulation, can contribute to the onset of metabolic syndromes, hence weight control is important in reducing risk of CVD.

Lipid-lowering therapy

Statins are medications administered to decrease the low-density lipoprotein (LDL) cholesterol and multiple trials have reported their benefit on the CV mortality and on the risk of major cardiovascular events[25]. The linear relationship between LDL-cholesterol and cardiovascular risk suggested that statins main benefit was related to the reduction of LDL-cholesterol. However, a number of additional effects of statins have been suggested to contribute to their efficacy in CVD. The Heart Protection Study reported that simvastatin reduced mortality and morbidity even in patients with 'normal' LDL-cholesterol levels[26]. Their potential benefit could also be explicated by improvements in endothelial function, halting or retardation of atheroma development, reduction in inflammation and antithrombotic effects[27].

Anti-Platelet therapy

The potential benefit of the anti-platelet therapy is related to the reduction of thrombus formation and vascular inflammation[28]. Aspirin has been widely studied for secondary prevention in patients with established atherosclerotic vascular disease. In addition, the combination of statin and aspirin are associated with the greatest reduction in mortality in a case-control analysis. Furthermore, other antiplatelet agents such as clopidogrel, prasugrel and ticagrelor have been introduced in the management of atherosclerosis showing encouraging results[29].

Anti-coagulation treatment

The coagulation cascade involves an interaction between the contact activation and the tissue factor pathway leading to the conversion of factor X to Xa, initiating the common pathway[30]. The subsequent conversion of pro-thrombin to thrombin, catalyzing the formation of fibrin, leads to the formation of a firm clot stabilized by aggregated platelets[31]. Initially, vitamin K antagonists, such as warfarin, were the only anticoagulant treatment widely available for human use. Approximately 65,000 patients are treated in US emergency departments annually for anticoagulant-related hemorrhagic events[32]. The high rate of adverse events requesting a strict monitoring of the patients stimulated the search for potentially safer medications. Targeting different steps of the coagulation process allowed the introduction of multiple novel anticoagulants such as direct thrombin inhibitors (e.g. dabigatran), and factor Xa inhibitors (e.g. rivaroxaban, apixaban)[33]. These have safer adverse events profiles.

Anti-hypertension treatment

Evidence from several RCTs report anti-hypertension medications, such as β-blockers, as effective in the reduction of recurrent acute myocardial infarction (AMI), sudden cardiac death and total mortality in patients with AMI[34]. Their beneficial effect is related to the reduction of the heart rate and blood velocity with a consequent lower flow turbulence and vascular wall stress. In addition, recent analyses support a positive effect of β-blockers on the progression of atherosclerosis[35]. Furthermore, renin-angiotensin system inhibition improves the endothelial function and RCTs show a reduction in coronary events not only related to a blood pressure reduction, but also a stabilizing effect on the atheroma[36].

Anti-inflammatory medications

Evidence of immune activation and cytokine signalling within atherosclerotic lesions support the inflammatory pathogenesis of atherosclerosis[37] supporting the role of inflammatory biomarkers as independent risk factors for acute CV events[38] and the involvement of LDL particles and their contents to activate innate and adaptive immunity[39]. Furthermore, animal models of atherosclerosis targeting the disruption of cholesterol-regulating genes leading to atherosclerosis report that interfering with immune signalling and inflammatory mediators has an anti-atherogenic effect[40-42]. Therefore, this evidence has encouraged the development of anti-inflammatory strategies for prevention and treatment of atherosclerosis targeting the inflammatory signalling cascades, pro-inflammatory cytokines, and inflammatory autacoids.

The JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial reported that statin reduced CV risk in patients with high levels of high-sensitivity C-reactive protein, a nonspecific inflammatory marker. This was also associated with a reduction in low density lipoprotein (LDL) cholesterol, leaving researchers in doubt of whether the clinical CV

benefit observed were related to the lipid lowering or than anti-inflammatory effect of statins[43]. Further support of the inflammatory nature of atherosclerosis came from a recent seminal intervention trial using Canakinumab. This is a human monoclonal antibody against interleukin-1β, inhibiting inflammation and down-regulating several late inflammatory markers. Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) was a randomized controlled trial in which 10,061 patients with a previous myocardial infarction and an elevated level of high-sensitivity C-reactive protein (2 mg or more per liter) received canakinumab at 3 different dosages or placebo every 3 months. After 48 months of follow-up the levels of hs-CRP and IL-6 were significantly reduced from baseline in the test group compared to placebo. At a certain dosage of canakinumab, the risk of the primary end point (nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death) was 15% lower than in the control group (3.86 vs. 4.50 events per 100 person-years)[44]. Similarly a low-dose methotrexate (a common anti-inflammatory drug used in Rheumatoid Arthritis) is currently being tested in a The Cardiovascular Inflammation Reduction Trial (CIRT) for the reduction of CV risk in patients with a previous myocardial infarction with diabetes or the metabolic syndrome[45].

IMPACT OF PERIODONTAL TREATMENT ON CARDIOVASCULAR EVENTS

ACUTE MYOCARDIAL INFARCTION (AMI)

AMI is an event of myocardial necrosis caused by an unstable ischemic condition[46]. Coronary artery disease (CAD) accounts for more than half of all cardiovascular events in the population under 75 years of age in the United States[47]. It is the consequence of the rupture or erosion of a vulnerable, lipid-laden atherosclerotic coronary plaque resulting in exposure of circulating blood to a highly thrombogenic plaque core and matrix materials[48].

The INTERHEART Study, a worldwide observational study across 52 countries and including 15,152 incident cases of AMI and 14,820 controls, identified 9 risk factors accounting for approximately 90% of the risk. These factors were smoking, lipids, hypertension, diabetes, obesity, diet, physical activity, alcohol consumption, and psychosocial factors[49,50].

The Periodontal Disease and the Relation to Myocardial Infarction (PAROKRANK) study compared the periodontal status of 805 patients who had presented with a first myocardial infarction with 805 controls less than 75 years of age. Alveolar bone loss was measured on a panoramic x-ray calculating the proportion of remaining bone height from the total root length and bone height around each tooth. According to the percentage of remaining bone the participants were divided in healthy (≥80%), mild to moderate periodontitis (from 79% to 66%), and severe periodontitis according (<66%). The odds ratio (OR) for first AMI for persons with PD in comparison with no PD was 1.49 (95% confidence interval, 1.21–1.83), and, after multivariable adjustment, it was 1.28 (1.03–1.60)[51]. Xu et al. in a recent systematic review and meta-analysis gathered the observational evidence on the association between AMI and PD pooling 22 observational studies (4 cohort, 6 cross-sectional and 12 case–control) accounting for 129,630 subjects[52]. The authors reported an increased risk of AMI in PD with OR of 2.02 (1.59-2.57). It is not clear what the attributable risk of periodontal disease is compared to the risk factors identified in the INTERHEART study[50].

Paju et al. recruited 141 patients hospitalized with acute non-Q-wave infarction or unstable angina pectoris. Dental data were collected to find out if periodontal status has a contribution to the outcome of the treatment. The study participants were randomized to either clarithromycin or placebo once a day for 3 months. The observed end points were CV death, myocardial infarction, unstable angina, or ischemic stroke during a follow-up period of the average of 519 days. The authors reported that long-

term clarithromycin treatment could be beneficial in prevention of recurrent CV events in patients not affected by periodontitis[53].

As of October 2018, there are no published data on the effect of PT on primary prevention of AMI. Periodontitis and Vascular Events (PAVE) pilot study is the only trial published on the effect of PT on the secondary prevention of cardiac outcomes[54]. The authors designed a pilot multicentre, parallel group, single-blind randomized controlled trial (RCT) enrolling 303 participants with PD who had ≥50% blockage of one coronary artery or have had a coronary event within three years of the trial. The participants were then randomized to receive either oral hygiene instruction, one regimen of full mouth scaling and root planing under local anesthesia (n = 151) or oral hygiene instruction and advised to discuss their oral condition with a dentist (n =152). The follow-up was 6 months to 25 months. Five patients in the test group and 7 in the control group reported CV events. There was no significant difference in cardiovascular events between patient undergoing periodontal treatment and community care with a risk ratio (RR) of 0.72 (0.23-2.22). It should be noted that this was a pilot feasibility study and was not powered to see an effect on CV events. The study did show the feasibility of carrying out such a RCT study in the future. The study also showed that in non-obese subjects the CRP levels were reduced in the treatment group.

Cross-sectional data of The Scottish Health Surveys from 1995 to 2003 pertaining 11, 869 men and women, mean of 50 years of age, was linked to a database of hospital admissions and deaths with follow-up until December 2007 (Information Services Division, Edinburgh). The study reported a total of 555 cardiovascular events over an average of 8.1 years of follow-up, of which 170 were fatal. Coronary heart disease was related to 74% of cardiovascular events. Participants who brushed less than once a day exhibited the highest incidence of cardiovascular events (HR of 1.7, 95% CI 1.3 to

2.3) when compared to those who brushed twice a day hinting that poor oral hygiene is related to a higher cardiovascular risk [55].

Due to the limited numbers of RCTs, additional evidence on the beneficial effects of PT on CV outcomes has been reported in cohort trials. In 2015 Lee et al. designed a retrospective nationwide, population-based study on a large cohort in Taiwan represented by 511,630 patients with PD and 208,713 controls monitored for the incidence rate (IR) of AMI from 2000 till 2010 using the Longitudinal Health Insurance Database 2000 (LHID 2000)[56]. The participants were defined as being affected by PD according to codes related to the need of periodontal treatment (ICD-9-CM codes 523.0–523.5) and they were followed from the cohort entry date until the date of hospitalization due to AMI (ICD-9-CM codes 410–412), death, or end of the study period in 2010. The study cohort was sub-divided in 3 groups according to the treatment they received during the follow up: a) dental prophylaxis (procedure codes: 91003, 91004), b) treatment consisting of subgingival curettage and root planning (procedure codes: 91006–91008) and/or periodontal flap operation (procedure codes: 91009, 91010) and/or tooth extraction (procedure codes: 92013, 92014) and c) no treatment.

The hazard ratio (HR) for AMI calculated with Cox regression models were 0.90 for the dental prophylaxis, 1.09 for the intensive group and 1.23 in the no treatment cohort. The authors adopted the same method to analyze the incidence of stroke in the cohort.[57] After adjustment for common cofounders, HRs of 0.78 (0.75-0.91) and 0.95 (0.91-0.99) were observed in the dental prophylaxis and intensive treatment groups compared to an HR of 1.15 (1.07-1.24) in the control of no treatment. Park et al. published data from the National Health Insurance System-National Health Screening Cohort, including 247 696 healthy adults, median 52 years of age, who had no history of major cardiovascular events with a median follow up of 9.5 years. 14 893 major cardiovascular events occurred including cardiac death, myocardial infarction, stroke, and heart failure (MACEs) and periodontitis was related to an increased risk of future major cardiovascular events. Furthermore,

brushing teeth one more time a day and regular professional cleaning were associated respectively with a 9% (HR: 0.91; 95% CIs: 0.89–0.93; P< 0.001) and 14% (HR: 0.86; 95% CIs: 0.82–0.90; P< 0.001) lower risk of cardiovascular events, independent of potential confounding factors or oral health problems [58].

Holmlund et al. followed a cohort of 8,999 patients with PD aged 20 to 85 years between 1979 and 2012[59]. The whole sample received nonsurgical and if necessary surgical treatment and after the active phase, all patients underwent a periodontal maintenance program. The authors then defined two groups: a) poor responders as having >10% PPD >4 mm and ≥20% sites with BOP 1 year after active treatment compared to b) good responders. After adjustment for age, sex, smoking, education, and calendar time, as well as for baseline values for BOP, percentage of PPD >4 mm, and number of teeth, poor responders had an Incidence rate ratio (IRR) of CVD of 1.28 (1.07-1.53) when compared with good responders suggesting that a successful PT might reduce the risk of CV events. These large insurance record studies suggest an effect of periodontal therapy on cardiovascular risk. However, they are not randomized controlled trials. To convincingly show that periodontal treatment reduced cardiovascular disease RCT's are needed.

STROKE

Stroke is defined as an acute episode of focal dysfunction of the brain, retina, or spinal cord lasting longer than 24 h or of any duration assessed by CT/MRI imaging or autopsy with focal infarction or haemorrhage relevant to the symptoms[60]. A TIA is considered a focal dysfunction of less than 24 h duration and with no imaging evidence of an infarction area.

According to the Global Burden of Disease Study 2010, an estimated 16.9 million incident strokes occurred between 1990 and 2010. There were 5·9 million deaths and 102 million disability-adjusted life years (DALYs) lost due to stroke, making stroke the second leading cause of death after ischaemic heart disease and third leading cause of DALYs lost worldwide[61]. Furthermore, the incidence of the first stroke has increased by 68% in recent years.

A meta-analysis of 2 cohort studies reported a 1.6-fold risk of stroke in presence of PD[62] and data from a recent meta-analysis of more observational trials described a RR of 2.88 (1.53–5.41)[63]. Wu et al., showed in a 21-year longitudinal study that periodontitis was related to ischemic stroke and not hemorrhagic stroke study specific for an atheromas process[64]. Chen et al. using the Taiwanese National Health Insurance Research Database and designing a retrospective cohort study, investigated the impact of PD on the onset of atrial fibrillation, one of most common cause of cardio-embolic stroke. They reported an increased risk of AF in the patients with PD compared to controls with a HR of 1.31 (1.25-1.36).

There is still lack of evidence and data from clinical trials on the effect of PT on primary and secondary stroke prevention. Sen et al. studied prospectively a cohort of 106 patients admitted to hospital with stroke or TIA [65]. The study participants received a periodontal assessment and according to CAL were divided in low periodontal disease group (LPD, lower 2 tertiles with the extent of attachment loss < 1.3%) and HPD (highest tertile with the extent of AL ≥ 1.3%). The cohort was followed for a median period of 24 months for the occurrence of vascular events such as stroke, AMI, and death. During the follow-up period 9 stroke, 7 TIAs, 3 MIs, and 8 vascular deaths were recorded. The HPD group had 5 strokes, 4 TIAs, 2 MIs, and 5 vascular deaths. Among the 66 patients with LPD, 11 had stroke, 7TIA, 1 MI, and 3 vascular deaths over the same period of follow-up. The finding suggested that in

stroke/TIA patients, HPD is independently associated with an increased risk of recurrent vascular events.

Observational data from a cohort study designed by Lee e al. suggested that PD is an important risk factor for ischemic stroke, and PT might lower the risk of stroke, particularly in younger age[57]. The authors designed a retrospective cohort study based on the Taiwanese National Health Insurance (NHI) Research Database administrative data to estimate the IR and hazard ratio HR for ischemic stroke among different treatments of PD during a 10-year follow-up period. 719 436 subjects. Ischemic stroke was recorded in 15 141 participants. Patients who underwent dental prophylaxis had a IR of stroke of 0.14%/year compared to 0.39%/year reported in those participants who had intensive treatment or tooth extraction group and 0.48% in the no treatment group. Further prospective evidence derives from the dental ARIC study in which the periodontal health of 6736 participants was assessed and 299 ischemic strokes over a follow-up of 15 years were reported[66]. Based on a questionnaire, the study participants were classified as regular or episodic in their dental care routine. Compared with a reference group of episodic dental care users, regular dental care users had a lower risk for ischemic stroke. After adjustment for race/center, age, sex, BMI, hypertension, diabetes mellitus, LDL level, smoking, and education, regular dental care use they were associated with lower rates of ischemic stroke (adjusted HR, 0.77; 95% CI, 0.63–0.94). These results are consistent with the epidemiologic study by Wu et al. that periodontal disease was related to ischemic stroke and not hemorrhagic stroke, consistent with the concept that periodontal disease effects the formation of atheromas.

PT AND TRADITIONAL CV RISK FACTORS

HYPERTENSION

Hypertension (HT) is a common traditional factor for CVD. HT denotes a persistent increase in arterial blood pressure (BP) denoted by either an increased systolic BP (SBP) greater than 130mmHg and/or diastolic BP (DBP), greater than 80mmHg[67].

Hypertension affects 30% to 45% of the general worldwide population[68] and it is a preventable risk factor for CVD. HT is also the leading single contributor to all-cause mortality and disability worldwide[69]. An increase of 20mmHG in systolic BP or a 10 mmHg in diastolic BP is associated with more than a doubling of the risk of stroke or ischemic heart disease mortality, whereas a reduction of 5 mmHg in systolic BP can decrease stroke mortality by 14% and CVD mortality by 9%[70].

Some evidence suggests a deteriorated periodontal status in patients with hypertension[71-74]. Several observational studies, mainly cross-sectional, with different definition of the exposure support an association between hypertension and periodontitis[75-86]. A recent meta-analysis reported an association between PT and HT with an OR of 1.50 (1.27-1.78)[87]. However the lack of proper experimental clinical evidence designed to investigate the effect of PT on BP as primary outcome, there is little or no evidence on the topic. All the data available has been extrapolated from intervention trials in which BP was included as a secondary outcome.

In normotensive patients, D'Aiuto et al. reported a reduction in systolic BP of 7±3 mmHg after periodontal treatment[88]. Furthermore, Graziani et al. in an pilot trial on the effects of PT on renal function described a significant reduction of 8.3 mmHg in systolic BP and 4.9 mmHg in diastolic BP[89]. However, in a different trial with a longer follow up and a different population the same group did not report reduction in either SBP and DBP[90] following PT. Vidal et al. in a pilot interventional study on PT and BP recruited 26 patients with refractory hypertension and generalized chronic periodontitis. All the participants underwent received oral hygiene, supra and sub-gingival scaling and then reevaluated 3 and 6 months after therapy. At 6 months follow-up, a significant reduction in

systolic BP [from 175 (38.8) to 157.5 (40)], diastolic BP [from 105 (21.3) to 95 (11.3)] was observed[91].

Houcken et al. designed a pilot intervention trial to investigate the effect of PT on pulse wave velocity (PWV) and BP. 45 participants received oral hygiene instructions and underwent supra- and sub-gingival debridement under local anesthesia. A subgroup (20 patients) was also randomly allocated to an adjunctive systemic antibiotic therapy with combination of Amoxicillin 375 mg and Metronidazole 500 mg for 7 days. BP was assessed at 3 and 6 months after treatment. DBP and heart rate did not show any significant change over time, however SBP was significantly reduced from 119.8 ± 14.6 to 116.9 ± 15.1mmHg[92].

Zhou et al. randomly allocated 107 patients with pre-hypertension and moderate to severe PD to receive either full-mouth scaling and root planing under local anesthesia or a standard cycle of supra-gingival scaling and polishing. The authors reported a reduction of systolic BP and diastolic BP levels by 10.26 and 7.21 mm Hg, respectively, 6-month following PT[93]. On the other hand, Higashi et al. did not show changes in both SBP and DBP after periodontal treatment in both otherwise patients with PD and in presence of HT[94]. Similar findings were reported by Seinost et al. in a normotensive population affected by severe PD 3 months following PT[95] and by Tonetti et al. in a RCT on otherwise healthy patients with severe PD[96].

It is clear that periodontal therapy may reduce blood pressure, however large RCT's are needed to convincingly demonstrate this,

ARTERIAL STIFNESS

The stiffness of the large central arterial system, such as the aortic tree, has been associated with systolic hypertension[97], coronary artery disease and stroke[98], heart failure[99] and atrial fibrillation[100]. Therefore, the arterial elastic properties have been adopted for CV risk stratification. Measurement of pulse wave velocity (PWV), as gold standard method for the assessment of arterial stiffness, has been recommended by the European Society of Cardiology as a tool to evaluate the arterial system damage, vascular adaptation, and therapeutic efficacy[68]. A meta-analysis of 10 observational studies concluded that PD is associated with an increased arterial stiffness expressed by a PWV mean difference of 0.85m/s (0.53-1.16)[101].

A limited number of intervention trials have reported the effect of PT on PWV. Vidal et al. reported an improvement in PWV [13.7 (2.4) to 12.5 (1.9)] 6 months after PT in hypertensive patients. Conversely, in 2 different trials PT did not lead to significant changes in PWV[92,102]. Kappellas et al. recruited 168 Aboriginal Australians in a RCT providing a single session of PT to the test group with no significant differences between treatments groups in PWV at 12 months follow up[102]. Similarly Houcken et al. did not detect a reduction in PWV after PT[92].

Jockel-Schneider et al. in an exploratory trial recruited 55 patients with severe periodontitis who underwent supra and sub-gingival mechanical debridement with or without the adjunctive of amoxicillin (500 mg) and metronidazole (400 mg) for 7 days. PWV values overall did not differ between baseline and 12 months follow-up (8.92 versus 8.85 m/s). However, the authors reported differences between the outcome tertiles based of bleeding on probing reduction (tertile 1—reduction in BoP ≥ 88% compared to baseline; tertile 2 < 88% to ≥64% and tertile 3 <64%) with a decreased PWV from 8.75 to 7.89 m/s in tertile 1[103]. Ren et al. randomized 108 patients with moderate to severe PD to receive either supra scaling and sub-gingival scaling and root planing or supra-gingival scaling and polishing. 1 month after PT the test group showed a significantly decreased PWV with a mean

difference of -0,58 m/s (-0.06-1.11)[104]. Again there is an indication that periodontal therapy may reduce pulse wave velocity, but large scale RCT's are needed to convincingly demonstrate this.

CIRCULATING SERUM BIOMARKERS

Lipid Profile

Assessment of lipid profiles and inflammatory markers such as C-reactive protein (CRP) and Interleukin-6 (IL-6) represents traditional and novel atherosclerotic risk markers and useful tools to improve CV risk assessment, monitoring of disease status, and response to treatment.

Several large clinical trials supported the involvement of cholesterol in the pathogenesis of atherosclerosis[105-107]. A reduction in Low-Density Lipoprotein (LDL)-cholesterol levels following statin therapy has beneficial effects on CV events. Evidence from a meta-analysis suggested that a 1mmol/l reduction in the level of LDL-cholesterol is associated with a 12% reduction in all-cause mortality, and a 19% reduction in CV mortality[108]. In addition, the lipoprotein–lipid profile other than LDL-cholesterol levels might have a reflection on CV risk[109]. A recent systematic review and meta-analysis of 19 observational trials with a total of 2,104 subjects reported significantly higher serum levels of LDL and triglycerides (TG) and lower High-Density-Lipoprotein (HDL) in PD[110]. However a previous meta-analysis of the intervention trails reported a non significant impact of PT on the lipid profile (HDL, LDL, TG, TC)[18].

A small RCT on 30 patients with hyperlipidaemia and chronic periodontitis on statin therapy evaluated the lipid profile 3 months following PT. The author reported a significant reduction of LDL in the test group[111]. Similarly, Fu et al in a RCT on 109 patients with PD and hyperlipidaemia

described lower levels of TG and higher HDL in the test group 6 months after PT[112]. A non RCT on the impact of obesity on the effect of PT recruited patients with generalized chronic periodontitis divided into 28 and 26 non-obese groups described a reduction of levels of TG, total cholesterol (TC) and LDL in the obese 3 months after therapy group failing to show the same effect in the control group[113]. In a RCT on 55 patient with CHD and PD, Hada et al. reported only an intragroup significant reduction of very Low-Density Lipoprotein (VLDL) in the test group 6 months following PT[114]. These early studies suggest an effect of periodontal therapy on lipids, however, large scale RCT's are needed to confirm these results.

C-reactive protein (CRP)

CRP is an acute phase response plasma protein. Acute phase reactants are molecules subject to increase or decrease by at least 25% of their plasma concentration in response to inflammation[115]. It was first described as a protein that reacting with the "C" polysaccharide of the pneumococcal cell wall[116] via phosphocholine ligand[117]. CRP has been adopted as a biomarker of disease activity in multiple conditions[118]. Apart from being an inflammatory marker, CRP explicates functions that may directly have an impact on the inflammatory response[119] interacting with various other ligands, activating the classical complement pathway, stimulating phagocytosis and binding to FcγR immunoglobulin receptors[120]. It is primarily secreted by liver hepatocytes[121], however, multiple extra-hepatic sources of have been identified but it is unlikely that the extra-hepatic production would substantially affect its serum profile[120].

CRP may increase 100 to 1000-fold within 72 hours of an inflammatory stimulus. Data from large observational studies suggest a wide variation of CRP levels in the general population with a mean of approximately 1.6 mg/L[122]. Generally the majority of the healthy population presents concentrations

lower than 1 mg/L; values equal or higher than 10 mg/L have been considered clinically important[123]. However, lower concentrations have been associated with the progression of osteoarthritis[124], rheumatoid[125] arthritis and the future CV risk[126]. The American Heart Association recommended the following concentrations as predictors of CV risk for the Western population: Low <1 mg/L, Average 1-3 mg/L, and High >3.0 mg/L[127].

Atherosclerosis is now regarded as an inflammatory condition[128]. The lipid and the inflammatory cells infiltration are observed in the earliest stages of plaque development[129]. However, adding the inflammatory profile to CV risk-prediction algorithms and clinical decision making does not provide a definitive advantage[130]. CRP might indicate low-grade inflammation linked to CV risk predicting CV morbidity and mortality, independent of established risk factors. Furthermore, CRP levels could be related to an increased waist circumference and sedentary lifestyle and consequent CV risk[131-133]. Data from an individual participant meta-analysis by The Emerging Risk Factor Collaboration (ERFC) suggests that CRP level has a continuous association with CV risk comparable to major lipid profiles and blood pressure[134].

The main question regarding the utility of CRP assessment in CV prevention is whether it is causal or a consequence of the disease. Mendelian randomization studies have investigated the associations between genetic variants of CRP, its plasma levels, and CV risk[135-137]. They show lack of consistency in the associations therefore reducing the evidence supporting a causal role of CRP. However, multiple trials support the inclusion of CRP profile for the risk prediction in primary prevention for both men and women[138,139]. The Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) has reported a significant CV risk reduction following statin therapy in asymptomatic individuals with high CRP, therefore CRP might be a biomarker to take into account in the CVD therapy[140].

PD could contribute to systemic inflammation and subsequent CV risk via elevation of the CRP profile. Data from a meta-analysis of observational trials supported elevated CRP levels in PD compared to controls with an estimated weighted mean difference (WMD) of 1.56 mg/l (95% CI 1.21– 1.90)[141]. The same group estimated the effect of PT on CRP profile in 2 non randomized controlled trials[142,143] reporting a between groups (treatment and controls) difference of 0.50 mg/L (95% CI 0.08 to 0.93)[141]. A previous meta-analysis based on 2 trials reported a non significant mean overall difference in CRP levels before and after treatment of 0.18mg/l (- 0.70 to 0.35 95% CI) in favor of PT[144]. The most recent meta-analysis on the topic dated 2013 included intervention trials with and without the addition of antibiotics suggesting a significant difference of 0.37 mg/L (95%CI=-0.64 to -0.11) between test and inactive controls (no periodontal treatment, OHI or supragingival cleaning) and no difference between test and active control groups[145]. The JUPITER trial reported that a reduction in median CRP values of 1.2 mg/L was associated with a 44% reduction in CV events after 12 months in subjects with a low risk lipid profile[43]. Therefore the effect of PT on CRP profile might be clinically relevant.

Very few trials have been published on the effect of PT on CVD patients and with limited follow-up[146,147]. A RCT on a 70 patients with coronary heart disease (CHD) and PD did not report statistically significant differences in CRP 6 months following PT[114]. In the general population multiple trials with different time points have been conducted. De Souza et al. in a small non-RCT reported a significant reduction of CRP 60 days after therapy only in patients with baseline levels of CRP>3 mg/L[148]. Gupta et al. in a non RCT described a reduction in CRP levels 3 months after periodontal treatment in patients with chronic and aggressive PD of 3.03±1.67 mg/L to 1.46±1.67 mg/L and from 3.09±1.21 to 1.43±1.21 mg/L respectively[149]. However Almaghlouth et al. in a non RCT did not find correlation between the CRP levels and periodontal parameters 3 months after PT[150] and Eickholz et al in a non

RCT did not report significant changes in CRP 3 months following PT in both chronic and aggressive PD[151]. Caúla et al. in a RCT on 64 patient with severe PD described a significant median difference [0.7 (0.51–0.83)] in CRP between tests and controls 6 months after PT[152]. In contrast, a RCT on a population with T2DM and PD did not report significant reduction in CRP 6 months after PT[153]. In addition, Ramich et al. in a non RCT evaluated the long-term effect of PT on CRP profile 5 years after PT describing a non significant difference in CRP compared to the baseline values[154].

In conclusion, based on the available evidence and limited by the lack of long term well designed RCT, PT seems to be associated with a mild reduction of CRP levels supporting its beneficial effect on the systemic inflammatory profile.

Interleukin-6 (IL-6)

Initially IL-6 was a cytokine believed to be mainly involved in the proliferation and activation of T cells, the differentiation of B cells, and the acute-phase response regulation[155-157]. It is now recognized as having a pleiotropic action with IL-6 acting in a wide range of homeostatic processes such as lipid metabolism, insulin resistance, mitochondrial activities, the neuroendocrine system and neuropsychological behaviour and systemic conditions such as CVD and rheumatoid arthritis[158-161]. IL-1β and tumor necrosis factor are its major activators, however other cytokines and pathways such as Toll-like receptors, prostaglandins, adipokines and stress responses can also trigger its expression. The physiological concentrations of IL-6 in human serum are normally in a range of 1 to 5 pg/ml, but in presence of disease it can reach a much higher concentration range (mg/l)[162]. Therefore, IL-6 levels can be associated with disease activity and often more predictably than CRP profile[163-165]. Evidence from Mendelian randomization trials does not support a causal role of CRP in CVD. A meta-analysis of nearly 200 000 participants reported a relative risk for CHD of 1.00 (0.90 to 1.13) per 1 SD higher

genetically raised CRP concentration[166]. However, genetic variants associate with higher circulating concentrations of IL-6 receptors and a lower IL-6 cell signalling seems to be protective against CHD[167,168]. This data have increased the interest in upstream key regulator cytokines in the development of atherosclerosis and the importance of inflammation in its pathogenesis. Some evidence suggests higher IL-6 concentrations in PD compared healthy controls and a reduced following PT[169,170]. However, a meta-analysis of RCT on the effect of PT on IL-6 concentration did not support an effect of PT on its serum levels[18]. In addition, recently Geisinger et al. conducted a RCT on 475 participants affected by Diabetes Mellitus Type 2 (T2DM) and PD without reporting a significant difference in the reduction of IL-6 6 months after PT between test and control groups speculating that T2DM rather than PD could mainly drive IL-6 levels[153].

SURROGATE VASCULAR IMAGING OUTCOMES

c-IMT

The assessment of the intima-media thickness of the carotid artery (c-IMT) by B mode ultrasound is a tool introduced to evaluate the presence and progression of atherosclerosis and estimate future CV risk. Being a non-invasive, safe and well tolerated exam contributed to its adoption in both clinical and research settings. The interest in c-IMT measurements derives from multiple observational studies. 1,257 male subjects of the Kuopio Ischemic Heart Disease Risk Factor (KIHD) study were followed for 2 years correlating c-IMT with the risk of myocardial infarction. There was an increased relative risk of 4.1 (95% confidence interval [CI] 1.8 to 9.2) in the presence of a plaque and if c-IMT was>1 mm there was a 2.1 fold risk of MI. Using c-IMT as a continuous variable led to 11% higher risk of MI each 0.1mm increase in c-IMT[171]. The Atherosclerosis Risk in Communities (ARIC) study observed a population of adults with no history of disease for 4 to 7 years. Having c-IMT > 1mm was

related to a hazard ratio of 2.62 (95% CI 1.55 to 4.46) in women and 1.20 (95% CI 0.81 to 1.77) in men after adjusting for CV risk factors[172]. However, the Rotterdam study reported a similar CV risk between genders[173]. Additionally, the investigators of the Carotid Intima Media Thickness [IMT] and IMT-Progression as Predictors of Vascular Events in a High Risk European Population (IMPROVE) study indicated that other parameters related to the common carotids, such as the increased intra-adventitia diameter can be predictors of CV events independently from the Framingham risk score[174]. c-IMT has also been associated with classic CV risk factors such as diabetes and hypercholesterolemia[175-177]. Hypertension is related to an increased c-IMT possibly due to a medial hypertrophy[178,179]. Interestingly, pharmaceutical interventions seem to have an impact on c-IMT.

Blood pressure and lipid lowering agents can reduce the progression of c-IMT or decrease its value[180-183]. Bogalusa Heart Study reported the presence association between c-IMT and CVD since young age[184]. The Pravastatin, Lipids, and Atherosclerosis in the Carotid Arteries II (PLAC-II) study reported 0.0295 instead of 0.0456 mm/year of progression between test and controls[185]. Rosuvastatin in the The Measuring Effects on Intima-Media Thickness: An Evaluation of Rosuvastatin (METEOR) study showed changes of −0.0014 and 0.0131 mm/year comparing test and placebo in a middle-aged populations with low Framingham risk scores (FRS)[186]. In addition the Monitored Atherosclerosis Regression Study suggested that changes in lifestyle such as smoking cessation and weight loss are associated with a 0.13mm/year reduction in progression of c-IMT. The progression rate ranges from 0.006mm/year in healthy participants to 0.06mm/year in patients with coronary artery disease[187]. In a post-mortem study, the increased aortic wall was related to mainly intimal changes. c-IMT augments 2 to 3 fold during life, however gender and ethnicity seems to partially explain a certain heterogeneity showing the highest values in Afro-Caribbean population and the lowest in Hispanics[188].

A systematic review and meta-analysis of observational trials reported a thicker c-IMT in PD[189]. In addition Zeng et al. in a systematic review and meta-analysis on 17330 participants showed that periodontitis is associated with carotid atherosclerosis (OR: 1.27, 95% CI: 1.14-1.41; P<0.001)[190]. Piconi et al. conducted an uncontrolled 12 months cohort clinical trial and concluding that periodontal therapy has a favourable effect on c-IMT progression. Due to its low level of evidence, however no firm conclusions can be drawn [191]. Kapellas et al. conducted a RCT on 168 Aboriginal Australians suffering from PD and observing a c-IMT decrease after 12 months of a single session of periodontal therapy in the intervention group (mean reduction=−0.023 [95% CI, −0.038 to −0.008] mm) but not in the control group (mean increase=0.002 [95% CI, −0.017 to 0.022] mm). The difference in intima-media thickness change between treatment groups was statistically significant (−0.026 [95% CI, −0.048 to −0.003] mm; P =0.03)[102]. However, the participants received a single session of periodontal therapy with a lack of SPT sessions and reported modest periodontal improvements. In addition, all participants were free to receive periodontal treatment during the course of the study. Furthermore two loops were obtained from each side of the carotid, and averaged to obtain the maximum carotid IMT increasing the variability of the measurements. The Oral Infections and Vascular Disease Epidemiology Study (INVEST) has documented that higher levels of periodontopathogens were cross-sectionally associated with thicker carotid IMT[192]. In addition, the same investigators reported longitudinal change in periodontal health to be concurrent with longitudinal carotid artery IMT progression over an average period of 3 years[193]. Desvarieux et al. detected a difference in c-IMT among participants of approximately 0.1 mm in 3 years follow-up suggesting the importance of the improvement in periodontal status. Evidence suggests that a 0.03 mm/year increase in c-IMT is associated with a 2.3-fold increased risk for CV events[194]. In addition, experimental studies on the impact of statins on the c-IMT progression rate reported as clinically significant a difference of 0.0082 mm/year in c-IMT between test and controls[185].

Endothelial function

A meta-analysis of 14 prospective studies reported a 13% lowering of future CVD per every 1% increase in FMD[195]. This finding corroborates the previous conclusion suggesting an improvement of vascular health not only in cases of severe, generalized periodontitis but also in moderate forms of periodontal infection[96].

A systematic review and meta-analysis reported a deterioration of FMD in PD and a beneficial effect of PT[189]. Further evidence supporting the beneficial role of PT in improving endothelial function derives from multiple RCTs; two were conducted assessing endothelial function via the response of brachial blood flow to acetylcholine injection and both reported a statistically significant improvement after periodontal therapy[94,196]. Our group in a RCT on otherwise healthy patients with sever PD concluded that 6 months after intensive PT the test group presented with a 2.0% greater value of FMD (95% CI 1.2 to 2.8; P<0.001) compared to controls[96]. In contrast, Saffi et al. have published the result of a RCT on PT and endothelial function assessed by FMD on 69 patients with coronary artery disease and severe PD who were randomly allocated to non-surgical PT or delayed treatment and reevaluated 3 months after[197]. The authors reported significant between-group differences in the endothelial function.

PT and ACUTE CVD RISK

The treatment of hyperlipidaemia and hypertension are classic examples of long-term CV risk management. However CV events often occur abruptly after plaque rupture and thrombosis[198] and certain activities/conditions such as heavy physical exertion, severe emotional stress, and respiratory infection have been identified as potential triggers of AMI, stroke or sudden cardiac death[199-201].

A within-person comparisons based on the United Kingdom General Practice Research Database investigated the risks of AMI and stroke after acute infections in 20,486 subjects with a first AMI and 19,063 subjects with a first stroke. The authors reported a higher risk (highest during the first three days) after a diagnosis of systemic respiratory and urinary tract infection supporting that acute infections are associated with a transient increase in the risk of CV events[201]. Therefore, a strategy to reduce the short-term risk, triggered acute risk prevention (TARP), might be beneficial in the management of CV events[202].

PD is a common source of local and systemic inflammation. We recently confirmed that the degree of inflammatory exposure periodontal therapy represents is linked to the extent and the duration of periodontal therapy performed[203].

Tonetti et al. reported that 24 hours after PT (full-mouth subgingival debridement with adjunctive minocycline microspheres), there are significant elevations of CRP, IL-6 and endothelial-activation markers soluble E-selectin and von Willebrand factor, indicating an acute systemic inflammatory response and a transient impairment of endothelial function[96]. Elevated levels of CRP and IL-6 in were also reported 1 day following PT (subgingival scaling within 2 days) in 60 patients with PD[151]. Similarly, Morozumi et al. analyzed serum samples 1 day following PT (full full-mouth subgingival debridement) reporting a statistically significant elevation in body temperature, CRP, interferon-γ and IL-12p70[204]. This transient systemic perturbation might be related to the bacteremia and local tissue trauma observed during invasive dental procedures[205,206].

Further, this evidence poses an additional question, which is whether acute inflammation following invasive dental treatments could be detrimental on patients' overall homeostasis and whether this

could be prevented. Our group reported on the possible association between dental treatment and acute increase in vascular risk. Minassian et al. adopted the self-controlled case series method to investigate the incidence of acute CV events such as ischemic stroke and myocardial infarction following invasive dental treatment by using Medicaid claims data from the United States. The authors reported "invasive dental procedures may be associated with a transient increase in the risk for stroke and myocardial infarction in the first 4 weeks after treatment"[207]. On the other hand, data from a nationwide population-based case-crossover study on 123,819 AMI patients and 327,179 ischemic stroke patients and a self-controlled design on 117,655 AMI patients and 298,757 ischemic stroke patients reported a non significant higher risk of AMI or ischemic stroke within the first 24 week after invasive dental procedures[208].

RATIONALE for a causal link between PD and CVDs

The current epidemiological findings of a moderate association between PD and atherosclerosis do not allow defining PD as an independent casual factor in the onset and progression of atherosclerosis. Bradford-Hill criteria have been adopted to define causation in disease models not responding to a single causal factor. Reviewing the modified Hill's criteria to explore causation, we acknowledge that PD satisfies the majority of the criteria (Table 1).

Table 2 Bradford-Hill criteria for the causal association between PD and CVD

Bradford-Hill Criteria	
Statistical strength of association	The strength of association between PD and CVD is considered weak to moderate.
Consistency	The association between PD and CVD is consistent among a large number of studies.

	However, consistency of the findings of available studies is not absolute.
Specificity	Positive association between periodontal and cardiovascular diseases was evidenced adjusting for traditional cardiovascular risk factors. Ischemic stroke but not hemorrhagic stroke as related to PD.
Temporal relationship	PD preceded CVD after adjustment for traditional cardiovascular risk factors.
Biological gradient	Increasing severity of PD resulted in higher cumulative incidence of CVD after adjusting for potential confounders.
Biological plausibility	Experimental evidence proves the biological plausibility of a causal association between PD and CVD.
Coherence	The association does not conflict with currently established theory and scientific knowledge on the development of CVD.
Experimental reversibility	*In vitro* and *in vivo* evidence supports a causal role for PD in the development of CVD. However, RCT on hard CV outcomes, ie MI and stroke, need to be designed.

Analogy	Other inflammatory conditions such as diabetes can induce CVD in humans. PD can induce CVD in animal models.

Despite the overall modest association, the consistency of data across different study populations, exposures and outcome variables suggests that these findings might not be spurious or attributable to confounders. There is lack of experimental evidence on the reversibility of the association obtained from systematic review of randomized controlled clinical trials testing the hypothesis that control of PD will result in a stop or reversion of atherosclerosis.

CONCLUSIONS

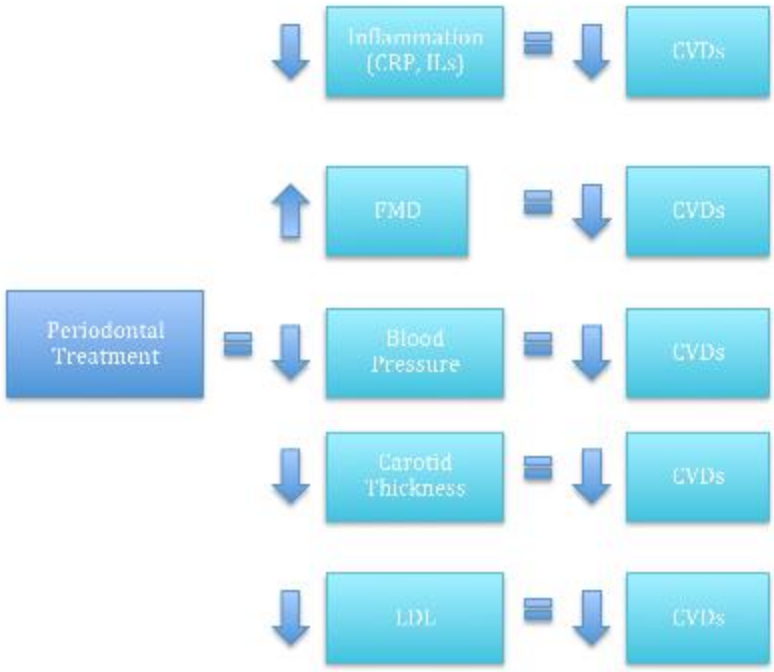
Evidence accumulated since 2013 further supports a potential contribution of infections to atherosclerosis-related inflammation. Multiple trials have been conducted to test the hypothesis that anti-infective therapies could be a tool in prevention of atherosclerosis and its complications. After initial data on the efficacy of *Chlamidya pneumoniae* (Cp) eradication in secondary prevention of cardiovascular events[209-213], large clinical trials, including several thousands of patients, have been performed. Particularly, the WIZARD (Weekly Intervention with Zithromax for Atherosclerosis and its Related Disorders), AZACS (Azithromycin in Acute Coronary Syndromes), ACES (Azithromycin and Coronary Events Study) and PROVE IT-TIMI 22 (Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial infarction) trials have investigated the effectiveness of different antibiotics in reducing CV risk in patients with ischemic heart disease, with or without serological evidence of Cp infection[214-217]. However, the results suggested a short-term benefit and no evidence of a long-term reduction of CV risk. Other trials focused on eradicating antibiotic therapy in patients with *Helicobacter pilory* (Hp) infection reporting in some cases a decrease of plasmatic

markers of CV risk and even an attenuation of restenosis phenomenon after percutaneous transluminal coronary angioplasty[218], while other studies reported no effects[219].

In conclusion:

- In the current available literature the main limitations of individual studies on the association between PD and CVD are the use of imprecise measures of PD, inadequate accounting for potential confounders such as unhealthy dietary pattern, premature birth, adverse life stresses, genetic predisposition and low statistical power for hard CV endpoints.
- PD and CVDs share many risk factors such as smoking, diabetes mellitus, increasing age, and poor socioeconomic conditions, obesity suggesting a possible common pathophysiology of PD and CVDs. This is supported by the identification of genes predisposing to both conditions (chromosome 9p21)[220].
- For the time being, it has not been yet clarified if PT can reduce the risk of CV events. The PAVE trial,it remains the only pilot study on this topic, and it was not powered to test the effects of PT and recurrent PD. Future fully powered RCT are needed
- For the time being, the available evidence suggests that PT has an impact on CV reducing multiple CV risk factors (Figure 2).

Figure 2 Impact of Periodontal Treatment on CVDs



A RCT designed to answer this question would require a large population (thousands of participants) to detect an effects of PT on CV events. Furthermore, considering the chronic nature of PD, a repeated intervals long-term treatment would need to be planned. In addition, an adequate follow-up length to detect CV events and an ethically acceptable control group would represent additional challenges to its feasibility. Many studies provide the rational and the PAVE study shows the feasibility of a well-diagnosed, fully powered study of PT and CVD.

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