

Evidence that periodontal treatment improves biomarkers and CVD outcomes

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D'Aiuto F, Orlandi M, Gunsolley JC. Evidence that periodontal treatment improves biomarkers and CVD outcomes.

Abstract

Aim: The aim of this review was to critically appraise the evidence on the impact of periodontal treatment of cardiovascular diseases (CVDs) biomarkers and outcomes.

Methods: A systematic search was performed in Cinhal, Cochrane, Embase and Medline for relevant articles up to July 2012. Duplicate screening and reference hand searching were performed. Data were then summarized and evidence graded in tables.

Results: The search resulted in: (a) no evidence on the effects of periodontal therapy on subclinical atherosclerosis, serum levels of CD40 ligand, serum amyloid A and monocyte chemoattractant protein-1, (b) limited evidence on the effects of periodontal therapy on arterial blood pressure, leucocyte counts, fibrinogen, tissue necrosis factor- α , sE-selectin, von Willebrand factors, d-dimers, matrix metalloproteinases, oxidative stress and CVD events, and (c) moderate evidence suggesting a negligible effect of periodontal therapy in reducing interleukin-6 and lipids levels, whilst a positive effect in reducing serum C-reactive protein levels and improving endothelial function.

Conclusions: Periodontal therapy triggers a short-term inflammatory response followed by (a) a progressive and consistent reduction of systemic inflammation and (b) an improvement in endothelial function. There is however limited evidence that these acute and chronic changes will either increase or reduce CVD burden of individuals suffering from periodontitis in the long term.

Key words: periodontitis; CVD; biomarkers; CRP; FMD; IMT; Inflammation; Oxidative Stress; Blood Pressure; Coagulation

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Cardiovascular diseases (CVDs), namely coronary heart disease (CHD), stroke, congestive heart failure, and peripheral artery disease, became the leading cause of chronic disease morbidity and mortality in industrialized countries in the twentieth century (Nabel 2003, Luepker

2011). CVD are now a global problem as their incidence is also increasing in the developing countries as a consequence of the better control of infectious diseases such as HIV, malaria and tuberculosis and due to the obesity and diabetes epidemic (Murray & Lopez 1997, Gersh et al. 2010). A large body of evidence is available on the beneficial effects of controlling a number of recognized CVD risk factor, including hypercholesterolemia, hypertension, smoking (Law et al. 1997, He et al. 1999, Kallio et al. 2010) and sodium

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intake (Strazzullo et al. 2009) and reduction of CVD mortality. However, the incidence of CVD is still increasing (World Health Organization 2007, Gersh et al. 2010) as controlling all recognized risk factors might not be sufficient at reducing CVD burden on the general population (Brunner et al. 2007, Law et al. 2009, Manktelow & Potter 2009).

Atherogenesis represents the main driver of CVD spanning over a number of decades and may eventually manifest with clinical event as myocardial infarction or stroke. Endothelial dysfunction is considered one of the earliest characteristics of atheroma progression. Indeed, the endothelium plays a key role in maintaining the normal function of the vessel wall. Several factors, such as hypercholesterolemia, oxidative stress, diabetes, cigarette smoking and infection, can increase expression of adhesion molecules and cause endothelial dysfunction. The increased production of adhesion mediators leads to an increased permeability of the intima and initial formation of atheroma (Ross 1993, Libby 2002).

There is emerging evidence that inflammation plays a key role in the development of CVD (Hansson 1999) from atheroma formation to its rupture and development of clinical events. Several epidemiological studies have investigated and support an association between high levels of inflammatory markers and increased risk and progression of CVD (Packard & Libby 2008). A number of potential sources of inflammation have been investigated over the last 30 years including bacterial and viral infections.

The possible aetiological role of acute or chronic infections on CVD has attracted great attention in recent years. In particular attention has been drawn on the potential impact of various infectious agents on systemic inflammation and autoimmunity and subsequently onset and progression of CVD (Kiechl et al. 2001).

Periodontitis is a chronic inflammatory disease affecting the periodontium and resulting in progressive attachment and alveolar bone loss (Armitage 1999). Its prevalence ranges from 6% to 50% of the population worldwide (Oliver et al. 1991, 1999 International International Workshop 1999, Eke et al.

2012). As most chronic diseases, periodontitis shares most of its putative and established risk factors with CVD, including age, gender, socioeconomic status, diabetes, obesity, smoking and hypertension (Friedewald et al. 2009). Evidence from prospective and cross-sectional studies supports a weak but consistent association between higher CVD risk and periodontitis (Bahekar et al. 2007). Individuals suffering from periodontitis on average presented with 14–15% greater risk of developing CVD from prospective trials, whilst the odds increased to more than 100% when analysing case-control studies compared to healthy individuals (Bahekar et al. 2007). A number of systematic reviews have confirmed these associations (Janket et al. 2003, Scannapieco et al. 2003, Khader et al. 2004, Lockhart et al. 2012).

A recent cohort study report on CVD among 1400 dentate men aged 60–70 years, showed that severe loss of periodontal attachment conferred a statistically significant doubled risk of death compared with controls (15.7% versus 7.9%) (Linden et al. 2012). The hazard ratio, adjusted for age, smoking, diabetes, hypertension, body mass index, cholesterol, education and marital status and history of a vascular event, was proved to be 1.57 (95% confidence interval (CI), 1.04–2.36). An increased mortality rate in individuals with a higher level of attachment loss had already been reported by other investigators (DeStefano et al. 1993, Garcia et al. 1998, Thorstensson & Johansson 2009). According to these observations, the potential benefit of periodontal treatment on CVD mortality could represent a novel therapeutic opportunity as to tackle CVD burden in the general population.

A recent position paper of the American Heart Association (AHA) reviewed the evidence available on the association between periodontal disease and CVD concluding that after 30 years of research, it is still not clear whether this link is causal (Lockhart et al. 2012). To date, only few systematic reviews have been published on the effects of periodontal therapy on CVD outcomes. This represents a challenging task for any reviewing group, as the large variety of study designs, interventions, multiple populations recruited (with/

without co-morbidities), length of follow-up, sample sizes would render almost impossible appraise and combine the published evidence under a single review. The recent AHA position paper systematically reviewed all the evidence on the epidemiological association between CVD and PD, describing the possible mechanisms involved but giving only a succinct description of all periodontal intervention trials published to date. The same paper called for new well-designed intervention studies investigating the impact of periodontal therapy on CVD outcomes. Due to the large number of small single-centre intervention trials published over the last 30 years focusing on various surrogate markers of CVD, the aim of this review is to critically appraise the evidence available on the impact of periodontal treatment on CVD biomarkers and outcomes.

Methods

Two investigators (M. O. and F. D.) performed the literature search restricted only to English language publications in the following databases:

- The Cochrane Oral Health Group's Trials Register (whole database).
- The Cochrane Heart Group's Trials Register (whole database).
- The Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library, current issue).
- MEDLINE via OVID (1950 to present).
- EMBASE via OVID (1980 to present).
- Cumulative Index for Nursing and Allied Health Literature (CINAHL) via EBSCO (1980 to present).

The search was carried out in July 2012. Examples of research strategies are included in Fig. 1. Then, we performed duplicate screening of all abstracts to identify relevant publications. Any disagreement over the eligibility of particular studies between investigators was resolved by reviewer consensus. As result of the search, 772, 207, 4143, 3849 articles in Cinhal, Cochrane, Embase, Medline were identified, respectively, and 585

Example 1

- #1 (periodontitis) or (periodontal disease) or (periodontal surgery) or (periodontal treatment) or (periodontal debridement)
- #2 (haemostatic factors) or (endothelial function) or (oxidative stress) or (LPS) or (MMPs)
- #3 (heat shock proteins) or (CD 40) or (microalbuminuria) or (IMT) or (blood pressure)
- #6 (lipid) or (acute phase reactant) or (cytokines) or (adhesion molecules) or (white blood cell)
- #7 (#6 OR #2 OR #3)
- #8 (#7 AND #0)

Example 2

- #1 (periodontal disease) or (periodont*) or (periodontal intervention) or (periodontal therapy) or (periodontal treatment)
- #2 (cardiovascular disease) or (atherosclerosis) or (thrombosis) or (cerebrovascular disorders) or (stroke)
- #3 (myocardial infarction) or (pulse-wave velocity) or (heat shock proteins) or (CD 40) or (microalbuminuria)
- #4 (Carotid IMT) or (blood pressure) or (haemostatic factors) or (endothelial function) or (oxidative stress)
- #5 (LPS) or (MMPs) or (lipid) or (acute phase reactant) or (cytokines)
- #6 (adhesion molecules) or (white blood cell)
- #7 (#1 AND (#2 OR #3 OR #4 OR #5 OR #6))

Fig. 1. Examples of search strategy.

potential relevant citations were selected. Comments, letters, editorials, news items, abstracts not followed by publication and consumer health material were excluded. Overall, 168 articles were selected based on their abstract. Finally, 76 were found to be suitable for this review based on relevance (Fig. 2).

Additional references were identified ($n = 17$) either through hand-searching reference lists from publications identified in the original search or through PubMed/electronic updates of journal table of contents. The same inclusion/exclusion criteria were used to screen these publications. Data were then summarized in evidence tables (Appendix S1–S9) and evidence grading was performed. The hierarchy of evidence was based on the classification scheme (U.S. Preventive Services Task Force et al. 1996), where I = evidence obtained from at least one properly randomized controlled trial (RCT); II-1 = evidence obtained from well-designed controlled trial without randomization; II-2 = evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one centre or research group; II-3 = evidence obtained from multiple time series with or without the intervention.

Dramatic results in uncontrolled experiments could also be regarded as this type of evidence; III = opinions of respected authorities, based on clinical experience; descriptive studies and case reports; or reports of expert committees. Authors

attempted to perform meta-analysis of data for relevant biomarkers of CVD following periodontal therapy. The following entrance criteria for studies to investigate high level evidence of RCTs were used:

- (1) RCT.
- (2) Control treatment without surgical or non-surgical therapy.
- (3) Active treatment included scaling and root planing (SRP), but could also include surgical therapy, extraction of hopeless teeth and/antibiotics.
- (4) Individuals had to have periodontitis, but the type of disease was not limited.
- (5) Follow-up time ranged from 6 to 24 weeks.
- (6) Studies had similar response variables.

After reviewing all clinical trials, authors agreed to perform meta-analysis only on markers where sufficient data were available (Table 1). One biomarker had been recently extensively reviewed and more than one systematic review with meta-analysis had been already published (Ioannidou et al. 2006, Paraskevas et al. 2008, Freitas et al. 2012). We therefore did not include it in a meta-analysis.

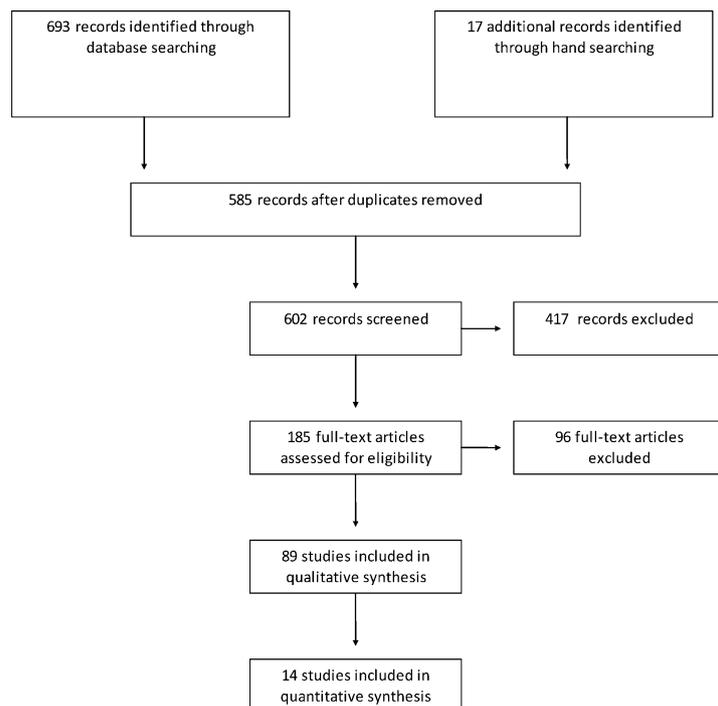


Fig. 2. Flow diagram of study inclusion.

This article was written according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Authors grouped CVD outcomes and biomarkers in traditional (lipids, blood pressure, and surrogate/hard endpoints) and novel (inflammation, coagulation and endothelial activation, plaque stability, and oxidative stress). After reviewing and grading all articles retrieved for each outcome, authors rated the strength of the overall scientific evidence according to the following scale (West et al. 2002, van Tulder et al. 2004):

- (1) Strong evidence (multiple relevant, high quality RCTs).
- (2) Moderate evidence (one relevant, high quality RCT and one or more relevant, low quality RCTs).
- (3) Limited evidence (one relevant, high quality RCT or multiple relevant, low quality RCTs).
- (4) No evidence (only one relevant, low quality study, no relevant RCTs or contradictory outcomes).

Results

Effects of periodontal therapy on traditional CVD risk factors

Lipids

Hyperlipidaemia is considered a well-established modifiable risk factor for CHD together with smoking, hypertension, glucose intolerance,

obesity, and physical inactivity. Specific lipid biomarkers have been identified. Triglycerides (TGs), serum total cholesterol (TC), and high/low-density lipoprotein cholesterol (HDL/LDL-C) are considered traditional lipid biomarkers associated with CVD, whilst recent surrogate markers include lipoprotein-associated phospholipase A2 (Lp-PLA2) and oxidized LDL (ox-LDL) (Boekholdt et al. 2004).

Numerous studies have demonstrated that lowering LDL-C levels can positively influence cardiovascular morbidity and mortality [Chapman et al. 2011, Third Report of the National Cholesterol Education Program (NCEP) Expert Panel 2002]. A meta-analysis reported that a 1.0 mmol/l reduction in LDL-C is associated with more than 20% reduction in CVD events (Baigent et al. 2010). Current guidelines refers to LDL-C concentrations that should be reduced to <2.6 mmol/l (100 mg/dl) in patients with established CVD and to <1.8–2.0 mmol/l (70–80 mg/dl) in those with very high CVD risk [Reiner et al. 2011, Third Report of the National Cholesterol Education Program (NCEP) Expert Panel 2002]. Similarly, reduction of TC to <4.5 mmol/l (174 mg/dl) is widely adopted as an effective strategy at reducing future CVD risk (Reiner et al. 2011). However, decreasing LDL-C levels to the recommended values, does not always abrogate the risk of having a major vascular event (Baigent et al. 2010).

Epidemiological studies have shown an inverse correlation between HDL-C and CVD events (Brewer 2004). Low serum concentrations of HDL-C are an independent risk factor for CVD. Levels <1 mmol/l (40 mg/dl), increase substantially the risk for CHD (Konush & Chapman 2006, Di Angelantonio et al. 2009).

Great attention has been given to changes in HDL-C as potential therapeutic targets for the treatment of CVD. Indeed, HDL-C is an essential regulator of the reverse cholesterol transport pathway: a process that allows excess cholesterol stored in peripheral cells, such as foam cells, to be excreted by the liver via the bile. This process is believed to protect against atherosclerosis (Chapman et al. 2010). However, HDL-C has multiple additional protective properties including its role in the reduction of LDL oxidation. ox-LDL, easily absorbed by the macrophage, contributes to the formation of foam cells (Navab et al. 2004) and therefore impact on the progression of atheroma. HDL-C also decreases vascular inflammation (Barter et al. 2004), thrombosis, improve endothelial function (Tso et al. 2006), promotes endothelial repair (Tso et al. 2006) and increases insulin sensitivity (Fryirs et al. 2010). HDL-C may also slow the progression of lesions by selectively reducing the production of endothelial cell adhesion molecules (CAMs) that facilitate the uptake of cells into the vessel wall (Barter et al. 2002)

Table 1. Summary of clinical studies included in evidence tables and assessed for meta-analysis

Appendix	Title	Outcome	Number of RCT's met criteria	Overall Level of evidence
S1	Effects of periodontal therapy on lipids	Lipids (multiple)	7	Moderate
S2	Effects of periodontal therapy on blood pressure	Systolic, diastolic	4 (two studies – data not shown, no difference)	Limited
S3	Effects of periodontal therapy on endothelial function	Endothelial Function – multiple measures)	3	Moderate
S4	Effects of periodontal therapy on white cell counts	WBC's	2	Limited
S5	Effects of periodontal therapy on acute-phase reactants [not C-reactive protein (CRP)]	Acute-phase reactions	3	Limited
S6	Effects of periodontal therapy on interleukins, /tumour necrosis factor-alpha, CD40/monocyte chemoattractant protein-1 and soluble adhesion molecules	Multiple cytokines and others	6 for interleukin-6	Moderate
S7	Effects of periodontal therapy on haemostatic factors	Multiple factors	2	Limited
S8	Effects of periodontal therapy on matrix metalloproteinases (MMPs)	Multiple MMPs	1	Limited
S9	Effects of periodontal therapy on oxidative stress	Multiple oxidative stress	2	Limited
	Effects of periodontal therapy on CRP	CRP	Paraskevas et al. 2008	Moderate

and bind/remove LPS (Murch et al. 2007). It therefore follows that increasing the concentration of HDL-C has the potential to reduce CVD risk.

In addition to HDL/HDL close link to atherogenesis, TG level is also considered an independent risk factor for CVD (Chapman et al. 2011). TGs are not directly atherogenic, but represent an important biomarker of CVD risk because of their association with pro-atherogenic proteins (Talayero & Sacks 2011). Lp-PLA2 has been proved to be an independent risk factor for CVD (Caslake & Packard 2003) as it can hydrolyse ox-LDL into pro-inflammatory mediators contributing to the atherogenic process.

In our search, we found 31 clinical trials investigating the impact of periodontal therapy on serum lipid levels (Appendix S1) of which 11 RCTs included seven trials of individuals with periodontitis alone and four trials involving individuals with periodontitis and other co-morbidities (i.e. diabetes, hypertension, metabolic syndrome, hypercholesterolemia). Two RCTs (D'Aiuto et al. 2005, 2006) examined a sample of otherwise healthy individuals affected by severe generalized periodontitis and showed that non-surgical periodontal treatment caused reductions in total and LDL-C levels at 2 and 6 months follow-up. In another RCT, Oz et al. (2007) performed periodontal treatment in 50 individuals also suffering from hypercholesterolemia and evaluated their serum lipid concentrations 3 months after the treatment. There was a substantial decline in TC and LDL-C profiles in the treatment group and between the two groups. Acharya et al. (2010) described changes in TG and HDL-C, just after 2 months of periodontal therapy in subject with metabolic syndrome. However, a number of clinical trials reporting no substantial differences in lipid profiles have also been found including individuals affected by periodontitis (Higashi et al. 2008, Kamil et al. 2011). Taylor et al. (2010) reported a statistically significant difference in TC after periodontal intervention compared to control. The most recent RCT reviewed was conducted in China on a sample of 134 individuals with diabetes with a 6 months follow-up (Chen et al.

2012). Participants were allocated to three different study arms; group 1 included patients undergoing non-surgical periodontal therapy at baseline and re-examined after 3 months, group 2 received treatment at 3 months follow-up, and group 3 of no intervention. Serum lipid concentrations were reported as decreased in all groups, but there were no statistical significant differences between the groups.

Two clinical trials (Montebugnoli et al. 2005, Tamaki et al. 2011) reported changes in ox-LDL after periodontal treatment. Montebugnoli et al. (2005) showed a mean reduction in ox-LDL of 18% in patients affected by periodontitis and CHD, whilst Tamaki et al. (2011) demonstrated a 37% reduction following periodontal therapy in otherwise healthy patients. In addition, Losche et al. (2005) found that the treatment of periodontitis could influence the serum activity of Lp-PLA2.

Further reports were found on the effect of periodontal therapy on the structure and metabolism of HDL-C. In three prospective studies (Pussinen et al. 2004a,b, Kallio et al. 2008), HDL-C concentrations were raised 3 months after periodontal therapy and also authors demonstrated significant anti-atherogenic profile (Pussinen et al. 2004c) suggesting indirectly that periodontitis could reduce the protective role of HDL-C on CVD onset. However, periodontal therapy did not seem to normalize HDL-C levels to low CVD risk.

More than one-third of the trials reviewed reported an improvement in serum lipid concentrations after periodontal therapy (reduction in TC in some and increase in HDL levels in other trials). This could represent a potential mechanism explaining the increased CVD risk in people with periodontitis (i.e. worsen cardio-metabolic profile of individuals suffering from periodontitis). If these associations were proven causal then periodontal therapy could be suggested as additional approach to lipid lowering medications in further reducing CVD risk of the general population. However, there is a wide variability among different trials outcomes reported. Factors such as age, gender, smoking status, general health status, medications, severity of periodontal disease and sample size should be taken into

account comparing outcomes from different studies. We attempted combining estimated effects of periodontal therapy on lipids from a number of RCTs. D'Aiuto et al. (2005) was included, but the control group was used twice once in contrast to standard therapy and once compared to intense therapy. Note that D'Aiuto et al. (2006) was not included, since there was no untreated control group. Other randomized trials that were not included were as follows: Pischon et al. (2007) (groups had SRP – contrast was use or none use of an antibiotic), Tüter et al. (2007) (a study of host modulation therapy with low-dose doxycycline, LDD *versus* SRP) and Payne et al. (2011) (only supportive periodontal therapy in both groups with one group receiving LDD).

The analysis was tested for heterogeneity which resulted in a statistically significant finding ($p < 0.048$ and I squared of 50.6). When one single study (Oz et al. 2007) was removed from the data, the heterogeneity reduced ($p < 0.65$, I squared of 0.00).

As noted in the forest plot, there did not appear to be any statistically significant results for any of the lipid markers tested (Fig. 3). We conclude that there is moderate evidence that does not support a positive effect of non-surgical periodontal therapy on lipid profiles.

Blood pressure

Hypertension is widely recognized to play a key role in the development of CVD events such as cardiac and renal failure, stroke and myocardial infarction (Whitworth 2003). The definition of high blood pressure is defined by a systolic blood pressure over 140 mmHg and/or a diastolic blood pressure over 90 mmHg in subjects who are not taking anti-hypertensive medication (Chalmers et al. 1999).

It has been hypothesized that the inflammatory chronic burden associated with periodontitis could have hemodynamic influences and therefore impact on the pathogenesis and progression of hypertension (Boos & Lip 2005). The hypothetical mechanisms include increased endothelial dysfunction and arterial stiffness. Our search found seven clinical trials examining the effect of periodontal therapy on blood pressure (Appendix S2).

Lipids

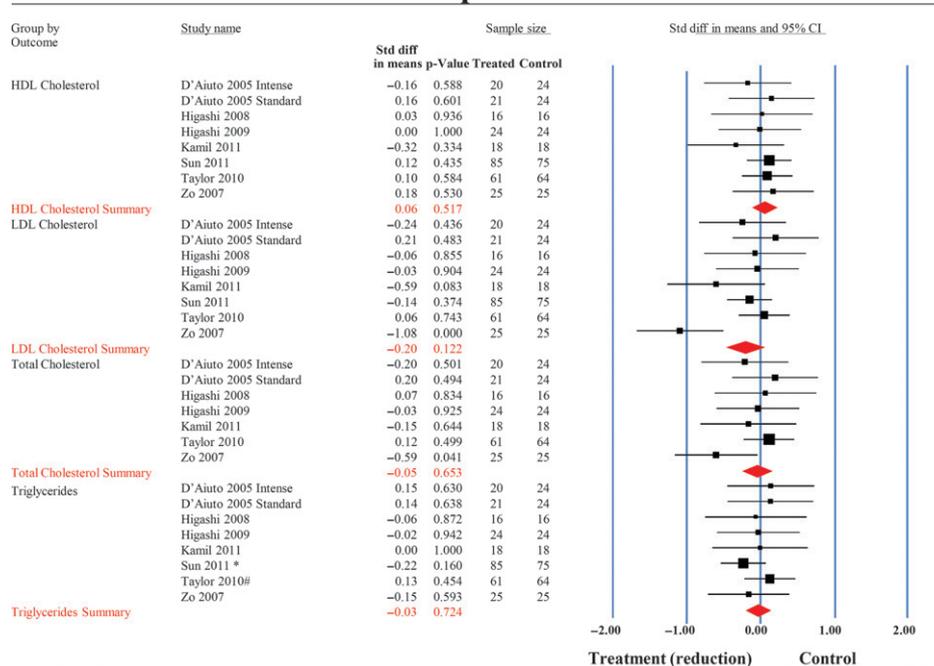


Fig. 3. Forest plot of periodontal treatment effect on serum lipids.

Seinost et al. (2005) reported no changes in blood pressure measures (diastolic/systolic) of 30 patients with severe periodontitis, 3 months after periodontal intervention. Conversely D'Aiuto et al. (2006) reported a reduction of systolic blood pressure 2 months after intensive periodontal treatment in patients affected by severe generalized periodontitis. In a subsequent trial, the same group failed to replicate these findings over a longer follow-up following non-surgical periodontal therapy (Tonetti et al. 2007). Higashi et al. (2008, 2009) in two RCTs reported not-statistically significant effects of periodontal treatment on blood pressure. Similar findings were reported in the remaining two trials (Graziani et al. 2010, Taylor et al. 2010). We summarize therefore that there is limited evidence on the effects of periodontal therapy in reducing systolic and diastolic blood pressure.

Effects of periodontal therapy on CVD surrogate and hard endpoints

Endothelial function

The endothelium is a key regulator of vascular biology including coagulation, inflammation and modulation of vascular growth and remodelling. The impairment of

endothelial function and integrity, called endothelial dysfunction, occur in the early stage of the atherosclerosis and its progression (Ross 1993, Gimbrone et al. 1995). Endothelial dysfunction can predict adverse CVD events and long-term outcomes (Schachinger et al. 2000). Flow-mediated dilatation (FMD) represents the most widely used non-invasive ultrasound method to assess endothelial function, it measures endothelium-dependent vasorelaxation of the brachial artery (Charakida et al. 2010).

A case-control study showed that otherwise healthy subjects with severe periodontitis had lower values of FMD compared to healthy controls (Amar et al. 2003). Seven intervention trials were identified describing the effects of periodontal therapy on endothelial dysfunction (Appendix S3); the majority of the trials was consistently associated with a positive effect of periodontal treatment on endothelial function (improvement) (Mercanoglu et al. 2004, Seinost et al. 2005, Elter et al. 2006, Blum et al. 2007, Tonetti et al. 2007, Higashi et al. 2008, 2009). Of the RCTs the largest was Tonetti et al. (2007) who randomized 121 healthy individuals suffering from periodontitis to either a cycle

of supragingival mechanical scaling and polishing (control) or full-mouth SRP, extraction of hopeless teeth and local delivery of microspheres of minocycline. Endothelial-dependent function was effected by periodontal therapy showing that 6 months after therapy there was an absolute difference of 2.0% (95% CI, 1.2–2.8; $p < 0.001$) between test and controls. Higashi et al. (2008, 2009) showed similar results in individuals suffering from hypertension and CVD, but used different measures of endothelial-dependent function. In summary, we report a consistent effect of periodontal therapy in improvement of endothelial-dependent function. The studies examined provide moderate evidence that periodontal treatment has a positive effect on endothelial-dependent function.

Subclinical atherosclerosis – carotid intima-media thickness

Carotid intima-media thickness (c-IMT) is a clinical surrogate measure of atherosclerosis (Hodis et al. 1996) and is also associated with established CVD risk factors (O'Leary et al. 1996) and outcomes (Chambliss et al. 1997, O'Leary et al. 1999). The IMT is the distance from the lumen-intima interface to the

media–adventitia interface of the artery wall, as measured by ultrasonographic images of the carotid arteries (not invasive). c-IMT, assessed by B-mode ultrasonography, can detect early morphological abnormalities of carotid artery walls that precede cardiovascular clinical events. However, c-IMT measurements are not strictly related to individual risk of cardiovascular events such as stroke or myocardial infarction (Touboul et al. 2012).

Evidence from observational studies suggests a moderate to strong association between periodontitis and c-IMT in otherwise healthy individuals (Beck et al. 2001, Leivadaros et al. 2005, Franek et al. 2006, Cairo et al. 2008, Li et al. 2009, Vieira et al. 2011).

The only published intervention study observing the impact of periodontal therapy on c-IMT was conducted in Italy by Piconi et al. (2009). Also, 35 otherwise healthy individuals affected by mild to moderate periodontitis took part in the trial without control group and received a scan of their carotids before and after periodontal therapy. An intra-group reduction of c-IMT at 6 and 12 months after the periodontal intervention was reported. So with only one study, there is no evidence on the possible effect of periodontal therapy on the rate of progression of c-IMT.

Cardiovascular mortality/morbidity

To date, no properly sized RCTs have systematically investigated the role of therapeutic periodontal interventions in the prevention of cardiovascular events. Only two trials can be mentioned (Paju et al. 2006, Couper et al. 2008). Paju et al. (2006) examined 141 individuals with acute non-Q-wave infarction or unstable angina pectoris enrolled in a double-blind, placebo-controlled study with the use of clarithromycin for 3 months. The average follow-up period reported was of 519 days (1.4 years). The rationale of the study was based on the assumption that antibiotic therapy could impact on the progression of chronic infections including periodontitis and therefore result in a reduction of CVD events rate. Antibiotic therapy however was not beneficial in preventing recurrent cardiovascular

events in periodontal patients compared to healthy subjects. The second trial is a feasibility multicentre, RCT designed to study effects of periodontal intervention on CVD secondary prevention. Study participants were randomized to either community care or SRP in University settings. Despite being only a feasibility trial, after 1 year of follow-up, no difference was observed in CVD events rate between the community control and the treatment groups (23 versus 24) (Beck et al. 2008). Recently reported in the AHA position paper, we confirm that there is limited evidence on the effects of periodontal therapy on CVD events including myocardial infarction or stroke (Lockhart et al. 2012).

Effects of periodontal therapy on novel CVD risk factors

Inflammatory markers

Inflammatory processes are recognized to play a central role in the pathogenesis of atherosclerosis and its complications (Ross 1993, Hansson 1999). Inflammation, focally and systemically, is involved in destabilization and rupture of atherosclerotic plaques, leading to acute cardiovascular events (Libby

2002). Current risk prediction models based on traditional risk factors have the ability to predict long-term CVD risk in many individuals. However, great effort has been given to research novel risk factors to further improve CVD risk prediction, with the aim also of detecting new targets for therapy and improve current prognostic algorithms (Wilson et al. 2005). A large number of inflammatory markers have been studied in this context, and the list is constantly growing. In Table 2, we report a number of novel CVD risk factors and at least one large prospective clinical trial that demonstrated its predictive value.

White blood cell count and differential. Circulating white blood cell (WBC) count represents a crude assessment of the individual inflammatory status (Hoffman et al. 2004) and has been proposed as a biomarker of CVD risk prediction (Pearson et al. 2004). A number of observational epidemiological studies have reported a consistent positive association between WBC count and the risk of CVD (Hansen et al. 1990, Kannel et al. 1992, Barron et al. 2001).

After our search we identified 15 clinical trials reporting the effect of

Table 2. Inflammatory serum biomarkers associated with the risk of coronary heart disease in prospective studies

Acute-phase reactants	High-sensitive C-reactive protein (Ridker et al. 1998) Lipoprotein-associated phospholipase A2 (Madjid et al. 2010) plasminogen-activator inhibitor 1 (Aso 2007) Serum phospholipase A2 (Boekholdt et al. 2005) Fibrinogen (Danesh et al. 1998) Serum amyloid A (SAA) (Johnson et al. 2004)
Leucocyte-derived enzyme	Myeloperoxidase (Meuwese et al. 2007)
Matrix metalloproteinases (MMPs)	Pregnancy-associated plasma protein A (PAPP-1) (Bayes-Genis et al. 2001) MMP-9 (Lubos et al. 2006) Tissue inhibitor of metalloproteinase-1 (Cavusoglu et al. 2006)
Soluble CD40 ligand	(Kinlay et al. 2004)
Cytokines	Interleukin-6 (IL-6) (Ridker et al. 2000) IL-10 (Heeschen et al. 2003) Monocyte chemoattractant protein-1 (MCP-1/CCL2) (Hoogeveen et al. 2005)
Cell adhesion molecules	Secretory intercellular adhesion molecule-1 (Hwang et al. 1997) Vascular cell adhesion molecule 1 (Hwang et al. 1997) sE-selectin (Hwang et al. 1997)
Vascular endothelial growth factor	Placental growth factor (Lenderink et al. 2006)
White blood cell counts	(Madjid et al. 2004)

periodontal therapy on WBC (Appendix S4). Christgau et al. (1998) failed to show any differences in WBC in patients with diabetes and healthy controls 4 months after periodontal treatment; these findings were confirmed in other trials (Montebugnoli et al. 2005, Seinost et al. 2005, Marcaccini et al. 2009a, Graziani et al. 2010, Taylor et al. 2010, Siribamrungwong & Puangpanngam 2012). In contrast, Christan et al. (2002) treated 27 periodontal patients showing that 3 months after therapy there was a statistically significant reduction in WBC and that the effect of the intervention was more evident in non-smokers when compared to current smokers. Similar results were obtained in two additional RCTs examining the effects of non-surgical intensive periodontal therapy on WBC after 6 months (D'Aiuto et al. 2006, Tonetti et al. 2007). Lalla et al. (2007) showed a reduction in percentage of mononuclear cells (CD14+ monocytes) following periodontal treatment. A recent report found that already 1 month after periodontal therapy individuals with CHD presented with a statistically significant reduction in WBC counts (Rastogi et al. 2012). That efficacy was further confirmed in three additional clinical trials (Fokkema et al. 2003, Hussain Bokhari et al. 2009, Piconi et al. 2009). Based on the evidence examined, only five trials reported a statistically significant reduction of WBC following periodontal therapy, whilst nine other trials reported the opposite finding. There is limited evidence on the possible effect of periodontal therapy on WBC.

Acute-phase proteins

Acute-phase reactants are molecules produced during acute and chronic inflammation; they exert a variety of functions including activation of complement factors, neutralization of bacterial pathogens and stimulation of repair and regeneration of a variety of tissues. These molecules are produced by the liver following a triggering stimulus (increased pro-inflammatory circulating cytokine levels) and have received great attention over the years and in particular C-reactive protein (CRP), plasminogen activator inhibitor 1 (PAI-1)

and fibrinogen (Blake & Ridker 2002).

C-reactive protein. C-reactive protein is a protein mainly produced by the liver but also by adipocytes and vascular smooth muscle cells and recently demonstrated in gingival tissues (Lu & Jin 2010) in response to a rise in interleukin (IL)-6 and tissue necrosis factor-alpha (TNF- α) (Calabro et al. 2003). CRP levels often increase substantially in response to a wide variety of biological insults, infections, inflammatory conditions and cancer (Pasceri et al. 2000). However, given its consistent association with CVD, CRP remains established markers of CVD risk, and it may very well be a contributor to the vascular inflammatory process in coronary arteries in humans. Multiple prospective cohort studies have established that increased CRP levels are associated with increased CVD risk in both genders, across a wide age range (Ridker et al. 1998, Koenig et al. 1999, Wilson et al. 2005). These findings have been consistent in different populations with diverse ethnic backgrounds and in diverse clinical settings, and CRP predicts a variety of CVD outcomes, including incident AMI, stroke, sudden cardiac death, stroke, peripheral artery disease and also incident diabetes and new onset hypertension (Pearson et al. 2004, Greenland et al. 2010). A recent meta-analysis showed that serum CRP concentration has continuous associations with CVD risk, ischaemic stroke and vascular mortality (Kaptoge et al. 2010). The risk ratio for CVD per 1-SD higher log(e) CRP concentration was 1.37 when adjusted for the conventional risk factors. Although CRP has multiple pro-inflammatory and pro-atherogenic properties, recent studies have not supported a causal role for it in atherogenesis (Casas et al. 2006). CRP is primarily a non-specific marker of inflammation, and its levels rise in response to infections, autoimmune diseases and malignant processes. In the absence of inflammation, hsCRP levels of 1 mg/ml confer a lower risk for CVD, whilst levels above 3 mg/ml almost double the risk of CVD (Madjid et al. 2004). Multiple measures known to reduce CVD risk

(i.e. smoking cessation, losing weight, exercise) also decrease serum CRP levels. Several medications, in particular, statins, are also known to reduce serum CRP levels (Ridker et al. 2008).

The association between CRP and periodontitis has been shown in several observational studies and at least three systematic reviews have examined the impact of periodontal therapy on CRP serum levels reduction. In the first meta-analysis, the weighted mean difference of CRP between cases with periodontitis and controls was 1.56 mg/l ($p < 0.00001$) (Paraskevas et al. 2008). This data confirm that diagnosis of periodontitis is associated with a state of low-grade systemic inflammation. In the same systematic review, data from six intervention studies were consistent with a 0.50 mg/l reduction of CRP serum levels after periodontal therapy (95% CI, 0.08–0.93) ($p < 0.02$). A second meta-analysis of intervention trials reported similar estimates with a mean overall difference in CRP serum levels after therapy of 0.2 mg/l (95% CI, -0.15–0.55) (Ioannidou et al. 2006). The most recent meta-analysis including four clinical trials reported a 0.23 mg/l reduction in CRP levels (-0.231; $p < 0.0001$) (Freitas et al. 2012). Two additional trials performed in individuals with periodontitis and other co-morbidities including diabetes (Sun et al. 2011, Chen et al. 2012) confirmed the potential anti-inflammatory effect of periodontal therapy also in these populations. Sun et al. (2011) randomized 157 individuals with diabetes to a periodontal intervention or control. After 3 months, the serum level of CRP in the treatment reduced from 5.81 ± 1.23 mg/l to 5.51 ± 1.29 with a mean difference of -0.30 ± 0.31 . Higashi et al. (2009) showed that 6 months after periodontal therapy individuals with CVD presented with a substantial reduction in CRP (from 2.7 ± 1.9 to 1.8 ± 0.9 mg/l) in the test group compared to the control. Similar results were reported in individuals affected by periodontitis and Metabolic Syndrome after 8 weeks of periodontal therapy (mean reduction of 0.68 mg/l) (Acharya et al. 2010). We can summarize therefore that there is moderate evi-

dence in support of a positive effect of periodontal therapy in lowering serum levels of CRP. Some studies have already reported a reduction of this marker to “normal” or low CVD risk levels following periodontal therapy (Paraskevas et al. 2008). There is also moderate evidence suggesting that periodontal therapy itself results in short lived systemic inflammation as measured by CRP levels; and this increase lasts up to 1 month after the therapy session (D’Aiuto et al. 2007).

Fibrinogen. Fibrinogen represents a major risk factor for CVD. Well-conducted meta-analyses have clearly shown that increased concentrations of fibrinogen are associated with the development or presence of atherothrombotic disease (Ernst & Resch 1993, Danesh et al. 1998, Fibrinogen Studies Collaboration 2004, Green et al. 2009). Strong associations (Bielak et al. 2000) between fibrinogen and coronary artery calcification and increased carotid intima-media thickness (c-IMT), both considered markers of subclinical coronary atherosclerosis, have also been reported (Baldassarre et al. 2008).

Seventeen trials ascertaining the impact of periodontal therapy on plasma levels of fibrinogen were found (Appendix S5). Six studies reported a reduction, whilst two reported an increase in fibrinogen levels following periodontal therapy. Hussain Bokhari et al. (2009) confirmed a positive effect of non-surgical periodontal treatment in reducing fibrinogen levels in both patients with CVD and periodontal patients who were systemically healthy. Indeed individuals affected by CVD, experienced a greater reduction in fibrinogen levels compared to healthy subjects. Similarly, Correa et al. (2010) showed a significant decrease in fibrinogen levels in subjects with periodontitis and type 2 diabetes, 3 months after periodontal intervention. Vidal et al. (2009) demonstrated similar findings but in people with periodontitis and hypertension. However, a larger number of investigators were not able to replicate these findings (Mattila et al. 2002, Montebugnoli et al. 2005, Lalla et al. 2007, Buhlin et al. 2009, Marcaccini et al. 2009a, Correa et al. 2010, Taylor et al. 2010).

Taylor et al. (2006) showed a significant decrease of fibrinogen levels 12 weeks after full-mouth tooth extraction. The same group failed to replicate this finding in a further trial with a non-statistically significant reduction in fibrinogen profile in the treatment group compared with the control group (Taylor et al. 2010). Some controlled clinical studies show reductions in fibrinogen levels, but RCTs failed to demonstrate reductions in this marker. Two investigations confirmed that periodontal therapy results in the short-term increase of fibrinogen levels (D’Aiuto et al. 2007, Graziani et al. 2010).

Thus, there is limited evidence supporting fibrinogen as a biomarker or being effected by periodontal therapy; in addition, the increase in the marker detected after periodontal intervention could be related to the treatment itself and therefore due to the short study follow-up designs reported, the chances of discovering a reduction in fibrinogen plasma levels were minimal.

Serum amyloid A. Serum amyloid A (SAA) is a systemic marker of acute and chronic inflammation. It also affects HDL composition and function (Clifton et al. 1985, Chait et al. 2005). SAA concentrations have been shown to positively correlate with the development of atherosclerosis and predict future CVD outcomes (Johnson et al. 2004, Chait et al. 2005). SAA serum levels have been reported to be raised in subjects affected by periodontitis and other co-morbidities (i.e. CVD), but in individuals with periodontitis only (Glurich et al. 2002). Vuletic et al. (2008) showed that 3 months after full-mouth extraction, serum levels of SAA were statistically significant lower when compared to pre-treatment (Appendix S5). Furthermore, Graziani et al. (2010) reported a bimodal response of SAA following periodontal therapy in a pilot study of 14 otherwise healthy individuals with generalized advanced periodontitis; SAA levels increased over the first few weeks after therapy, but then 6 months later they were statistically significant lower than baseline. Pussinen et al. (2004c) however showed no differences in SAA levels in 30 otherwise healthy subjects with periodontal

disease 3 months after periodontal treatment.

In summary, there is no evidence supporting a potential impact of periodontal therapy on SAA serum levels.

Cytokines

Cytokines are small soluble proteins that transfer information from one cell to another. More than 200 cytokines have now been identified including ILs, growth factors, chemokines and interferons. They are all organized in complex networks playing fundamental roles in both pro and anti-inflammatory processes including CVD and periodontitis.

Several studies showed that individuals with periodontitis have higher concentrations of circulating pro-inflammatory cytokines when compared with controls (Gorska & Nedzi-Gora 2006). In addition, subjects with both CVD and periodontal disease show significantly higher concentrations of cytokines when compared to individuals with CVD only (Higashi et al. 2009). Substantial evidence highlights the potential relevance of cytokines in mediating the inflammatory processes during atherogenesis and the development of CVD (Ridker et al. 2000, Heeschen et al. 2003) and several studies investigated the efficacy of periodontal intervention on cytokine serum concentrations (Appendix S6).

Interleukins. Interleukins are secreted proteins that bind to their specific receptors and were first identified to play a role in the communication among leucocytes but now it remains known that they play this role between all cell types. ILs is a large family that comprises about 37 subgroups; among them the most studied because of their strong association with CVD are IL-1, -6, -8, -10 and -18.

Cytokines in blood are mainly bound to other proteins such as auto-antibodies, receptor fragments and α 2-macroglobulin. The most widespread technique utilized to assess cytokines concentration in biological fluids is the sandwich enzyme-linked immunosorbent assay (sandwich ELISA) (Mire-Sluis et al. 1995). Such test has been proven to

detect only a small fraction of the whole cytokine level since it can identify the unbound fraction only and lack of discrimination between biologically active and inactive molecules (Malone et al. 2001). Therefore, it is not possible to determine accurately the profile of several cytokines in blood.

The pro-atherogenic effect of IL-1 is attributed to its ability to modulate a number of key events involved in the complex inflammatory process of atherogenesis such as the vessel wall inflammation, leucocyte chemotaxis and adhesion or plaque rupture (Bochner et al. 1991, Libby et al. 1995, Waehre et al. 2004). IL-6 is a key pro-inflammatory and pro-coagulant cytokine involved in triggering a systemic inflammatory response and also exerting hormone-like actions (Willerson & Ridker 2004). It can increase plasma concentrations of fibrinogen and PAI-1 (Devaraj et al. 2003), as well as increase in the hepatic expression of CRP (Willerson & Ridker 2004). IL-8 is a pro-inflammatory cytokine produced by various cell types involved in atherosclerosis, including endothelial cells, peripheral blood monocytes and vascular smooth muscle cells. The role of IL-8 in atherosclerosis could be mediated through its chemoattractant and mitogenic effects on vascular smooth muscle cells (Yue et al. 1994). In addition, IL-8 plays an important role on the infiltration of monocytes into the sub-endothelial space, which represents a crucial step in the early stages of atherosclerosis (Gerszten et al. 1999). Serum levels of IL-10, a potent anti-inflammatory cytokine, have recently been shown to be reduced in patients with acute coronary syndromes (Smith et al. 2001), thus suggesting that the absence of this cytokine may favour plaque instability and the development of acute coronary syndromes. IL-18 is a pleiotropic pro-inflammatory cytokine which plays an important role in the inflammatory cascade (Gracie et al. 2003) and potentially on atherosclerotic plaque progression and vulnerability (Gerdes et al. 2002). Circulating concentrations of IL-18 have been prospectively associated with vascular events in patients with stable and

unstable angina (Blankenberg et al. 2002) or pre-existing CVD (Blankenberg et al. 2003, Tziakas et al. 2007).

Several trials analysed the impact of periodontal therapy on various ILs serum levels (Appendix S6).

In two RCTs from the same group, otherwise healthy individuals suffering from periodontitis showed a reduction in IL-6 after periodontal therapy (D'Aiuto et al. 2004, 2006). These results are in line with a subsequent report showing reductions in IL-6 levels in subjects affected by periodontal disease and CVD (Higashi et al. 2009). Similar findings for IL-6 were shown in individuals suffering from periodontitis and co-morbidities like type 2 diabetes (Sun et al. 2011), hyperlipidaemia (Fentoglu et al. 2010) and refractory arterial hypertension (Vidal et al. 2009). Buhlin et al. (2009) reported significant changes in IL-18 12 months after periodontal therapy, but not in other ILs (-1 β , -4, -5, -6, -8, -10). A number of clinical trials reported no efficacy of periodontal therapy on ILs profiles (Lalla et al. 2007, Pischon et al. 2007, Correa et al. 2010) and these are summarized in Appendix S6. The most consistent finding was a reported increase of IL-6 levels following periodontal therapy in the short term (1 month) and some evidence of a reduction in IL-6 levels in the medium terms (6 months). After combining six RCTs that included in a consistent manner IL-6 as outcome

in a meta-analysis, we found no effect of periodontal treatment in reducing IL-6 serum levels (Fig. 4). The analysis was tested for heterogeneity (p -value of 0.66, Q -value of 4.9, and I squared of 0.00) confirming a consistent lack of effect of periodontal therapy. We conclude that there is moderate evidence that does not support an effect of non-surgical periodontal therapy on serum IL-6 levels.

Tumour necrosis factor-alpha. Tissue necrosis factor-alpha is a significant independent predictor of CVD events and total mortality among men (Tuomisto et al. 2006). It is regarded as a pivotal pro-inflammatory cytokine but it can be detected in many human atheromas (Barath et al. 1990). It is produced in murine and in human atherosclerotic lesions, primarily by macrophages/foam cells, activated T-cells, smooth muscle cells, and endothelial cells (Reckless et al. 1999). At the local level, TNF- α has the potential to promote cellular infiltration to the plaque via endothelial activation (Berk et al. 2001) and may induce endothelial dysfunction (Picchi et al. 2006). It also promotes the production of other cytokines as well as chemokine expression (Zhao et al. 2003), the expression of matrix metalloproteinase (MMP)-9 (Saren et al. 1996), hence increasing plaque instability. TNF- α can also promote angiogenesis (Leibovich et al. 1987). Its kinetics (early rise and fall) after an

Forest Plot of Periodontal Treatment Effect on serum IL-6

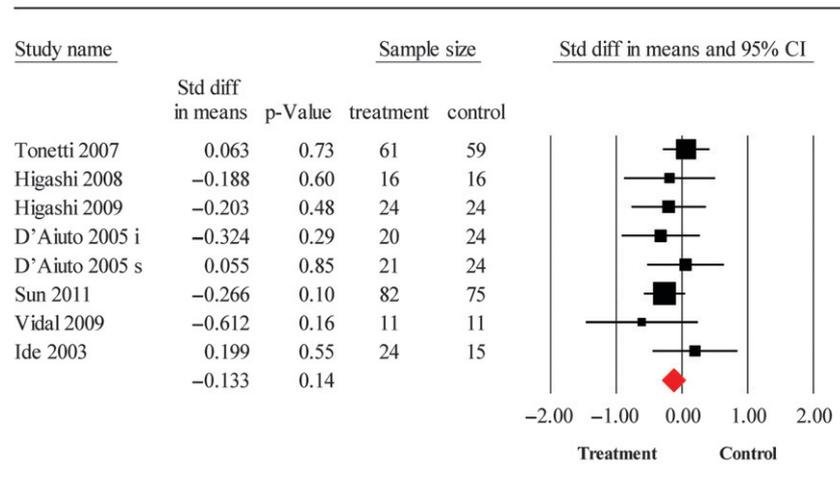


Fig. 4. Forest plot of periodontal treatment effect on serum interleukin-6.

inflammatory stimulus renders TNF- α not a very stable biomarker with basal levels often close to or lower than the commercial assays detection levels.

Individuals with periodontitis showed higher TNF- α serum concentration compared with controls (Gorska & Nedzi-Gora 2006). However, we found only five trials reporting on the effect of periodontal therapy on serum levels of TNF- α . Iwamoto et al. (2001, 2003) showed a significant reduction in TNF- α serum levels when comparing periodontal therapy and minocycline *versus* control. These results are in line with other three clinical studies (Correa et al. 2010, Duarte et al. 2010, Sun et al. 2011) describing the effect of periodontal therapy in individuals suffering also from other co-morbidities including diabetes. An equal number of trials failed to show a statistically significant effect of periodontal therapy on TNF- α (Ide et al. 2003, Lalla et al. 2007, Kallio et al. 2008, Buhlin et al. 2009, Fentoglu et al. 2011, Chen et al. 2012). Lastly, Fokkema et al. (2003) investigated the long-term effect of full-mouth tooth extraction therapy on the responsiveness of peripheral blood monocytes in a single subject with generalized terminal adult periodontitis reporting no changes in TNF- α release before and after therapy.

We conclude that there is limited evidence on a short-term increase of TNF- α levels following periodontal therapy, but no evidence on the long-term effects (reduction *versus* no effect).

CD40L/CD40. CD40 ligand is a member of the TNF superfamily, and interacts with CD40 (a TNF receptor superfamily member). Increased levels of sCD40L have been found in a range of chronic diseases, including CVDs, diabetes, peripheral arterial disease, pulmonary hypertension, acute/chronic heart failure, and stable/unstable angina (Aukrust et al. 1999, Blann et al. 2005). CD40L has been investigated as a prospective risk marker of CVD (Kinlay et al. 2004).

Marcaccini et al. (2009a) reported the effects of periodontal therapy on CD40L levels in 20 controls and 25 individuals with periodontitis. At

baseline, a statistically significant difference in the serum concentrations of CD40 ligand between the two groups ($p = 0.009$) was detected suggesting that the CD40 ligand may be increased in patients with periodontitis. However, no differences in CD40 ligand concentrations were found in the periodontitis group ($p = 0.41$) 3 months after therapy.

We conclude that there is no evidence on the effect of periodontal therapy on CD40 ligand.

Circulating cell adhesion molecules

Vascular (V)CAM-1, intercellular adhesion molecule (ICAM)-1, and sE-selectin are expressed by endothelial cells, macrophages and smooth muscle cells in response to various inflammatory stimuli (shear stress, oxidative stress, microbial stimulation, or inflammatory mediators) (Davies et al. 1993). Adhesion molecules are also involved in the adhesion and transmigration of leucocytes into the vascular endothelial wall thus promoting atheroma plaque growth and instability. Adhesion molecules are considered markers of vascular stress and their role in the pathogenesis of CVD has been extensively investigated (Libby 2002). Selectins (CD62) promote transient rolling of leucocytes along the endothelium, whereas ICAM-1 (CD54) and VCAM-1 (CD106) mediate attachment and transendothelial migration of leucocytes (Adams & Shaw 1994). In particular, VCAM-1 is linked to future CVD death (Blankenberg et al. 2001).

A number of studies indicate up-regulated adhesion molecules expression in periodontal lesions and increased circulating levels when periodontitis is diagnosed (Beck & Offenbacher 2002). The impact of periodontal treatment on these expression/levels has also been investigated. We retrieved evidence from six different clinical trials. A statistically significant reduction of soluble sE-selectin levels after periodontal therapy was reported in some of them (D'Aiuto et al. 2007, Pischon et al. 2007, Tonetti et al. 2007). No evidence of a similar effect of periodontal therapy on sICAM-1 and sVCAM-1 was found (Lalla et al. 2007, Pischon et al. 2007, Marcaccini et al. 2009a). We found only one

trial reporting a reduction of sICAM-1 after periodontal therapy in patients with low CVD risk (Rose-Hill et al. 2011).

In summary, we found limited evidence of a short-term increase and medium-term reduction in sE-selectin levels after periodontal therapy but not for other soluble cell adhesion molecules.

Monocyte chemoattractant protein-1. Monocyte chemoattractant protein-1 (MCP-1), a member of the CC chemokine family, is involved in the pathogenesis of atherosclerosis by promoting recruitment of inflammatory cells into the vessel wall (Rossi & Zlotnik 2000, Hoogeveen et al. 2005).

Serum MCP-1 concentrations have been found raised in individuals with periodontal diseases (1.5-folds in gingivitis, threefolds higher in individuals with periodontitis) when compared to healthy controls (Pra-deep et al. 2009). Further Fokkema et al. (2003) reported a twofold reduction of serum MCP-1 after dental clearance in a case report. Despite this encouraging data, three clinical trials comparing serum MCP-1 concentration before and after periodontal therapy failed to show any statistically significant reductions (Lalla et al. 2007, O'Connell et al. 2008, Marcaccini et al. 2009a).

We therefore conclude that there is no evidence that periodontal treatment effectively reduce MCP-1 levels.

Haemostatic factors (Appendix S7)

Plasminogen activator inhibitor 1. Plasminogen activator inhibitor 1 belongs to the family of serine protease inhibitors and is produced in high quantity by a number of cells including endothelial cells and hepatocytes in response to inflammatory cytokines. Raised PAI-1 plasma levels are consistently found in individuals with severe sepsis but also with other acute or chronic inflammatory diseases such as atherosclerosis (Aso 2007, Iwaki et al. 2012). PAI-1 is up-regulated by inflammatory cytokines and may therefore be regarded as a marker for an ongoing inflammatory process (Binder et al. 2002). Increased plasma levels of PAI-1 are positively correlated with the risk of developing

CVD as well as with the extent of coronary sclerosis, restenosis, risk of myocardial infarction and deep vein thrombosis (Kohler & Grant 2000).

Montebugnoli et al. (2005) reported for the first time that individuals with periodontitis presented with raised PAI-1 levels. Taylor et al. (2010) showed significant reductions in PAI-1 and 3 months after full-mouth extraction in people with terminal dentition due to periodontitis. However, the same group, in a RCT of periodontal therapy could not replicate the same findings (Taylor et al. 2010). Tonetti et al. (2007) also showed that periodontal therapy did only increase acutely PAI-1 24 h following periodontal therapy, whilst no greater reductions were observed 6 months after treatment. In addition, Lalla et al. (2007) treated 10 individuals suffering from diabetes and moderate/severe periodontitis and analysed PAI-1 levels 1 month after full-mouth subgingival debridement. They showed no statistically significant difference for this marker before and after treatment.

Thus, there is currently limited evidence to support the effect of periodontal therapy on PAI-1 although some studies support an increase rather than decrease of this marker in the short term.

D-dimer. D-dimer is a marker of coagulation and specifically for cross-linked fibrin turnover as it is derived from fibrin degradation. In coagulation disorders, the D-dimer profile can be severely altered with increased expression and a strong association with CVD (Lip & Lowe 1995, Lowe & Rumley 1999, Danesh et al. 2001). D-dimer plasma levels are also considered a strong predictor of coronary artery disease (Lowe et al. 2004).

Periodontal therapy performed in 18 males aged 40–65 years suffering also from CVD, did not produce a statistically significant reduction in D-dimer (Montebugnoli et al. 2005). On the contrary two reports showed a significant increase of D-dimer profiles 1 week after an intensive session of subgingival periodontal therapy and a subsequent decrease to baseline value within 1–3 month (D'Aiuto et al. 2007, Graziani et al. 2010).

There is therefore limited evidence in support of an acute effect of periodontal therapy on D-dimers in the first month following treatment but no effect in the medium-long term.

von Willebrand factor. von Willebrand factor (vWF) is an endothelium-released glycoprotein produced in the liver (Mannucci 1998). It has been proposed as a valid biomarker of endothelial damage/dysfunction. Indeed, raised plasma concentrations are often observed in inflammatory and atherosclerotic vascular diseases (Blann & Lip 1998). High plasma vWF levels (≥ 221 IU/dl) represent an independent risk factor for CVD events (Roldan et al. 2011).

Higher vWF antigen levels were reported in individuals with a poor dental status with acute myocardial infarction and healthy controls (Mattila et al. 1989). Furthermore, a more recent report confirmed increased vWF concentrations in individuals with severe periodontitis when compared to healthy controls (Leivadaros et al. 2005).

A small number of intervention trials reported no substantial reduction in vWF, 3 months after periodontal therapy (Montebugnoli et al. 2005, Taylor et al. 2010). Two clinical trials showed instead a statistically significant increase of vWF levels within 1 month after intensive periodontal treatment (D'Aiuto et al. 2007, Tonetti et al. 2007).

There is therefore limited evidence on the effects of periodontal therapy on vWF in the medium-long term, suggesting that periodontal therapy may increase vWF levels in the short term.

Matrix metalloproteinases

matrix metalloproteinases are a family of zinc-containing endo-proteinases that have all similar structural domains, but differ in terms of substrate specificity, cellular source, expression and regulation. The metabolism of the extracellular matrix is governed by a fine balance between the MMPs and their endogenous inhibitors, tissue inhibitor of metalloproteinase (TIMPs) (Sternlicht & Werb 2001). Two of the MMP related biomarkers most consistently implicated in CVD development and prognosis are MMP-9 and

TIMP-1 (Sundstrom & Vasan 2006). Circulating levels of these MMPs have been related to most CVD risk factors in large community-based samples (Sundstrom et al. 2004, Hansson et al. 2009) and have been associated with risk of death in patients with known CVD (Lubos et al. 2006, Hansson et al. 2011).

Our search identified five clinical trials reporting on the effect of periodontal therapy on MMPs (Appendix S8). Higher serum circulating levels of MMP-3, -8, -9 were detected in patients with periodontitis when compared to healthy individuals, whilst no difference was found in MMP-2 and TIMP-1, -2 levels (Marcaccini et al. 2009b). In the same study, levels on MMPs were analysed 3 months after non-surgical periodontal treatment; a statistically significant difference was found in MMP-8 and MMP-9, whilst no effects were shown on MMP-2, -3 and TIMP-1, -2 levels (Appendix S8). Combined effects of periodontal therapy with adjunctive low-dose tetracycline therapy on MMPs have been shown in multiple studies (Gorska & Nedzi-Gora 2006, Lalla et al. 2007, Payne et al. 2011, Koromantzios et al. 2012). Gorska et al. (2006) compared conventional periodontal treatment alone or in combination with a LDD and showed a not-statistically significant reduction in MMP-9 concentrations and increase in TIMP-1 in both treatment groups. Two further reports showed not significant trend in MMP-9 reduction (Lalla et al. 2007, Koromantzios et al. 2012). Payne et al. (2011) randomized 128 post-menopausal women with periodontitis to either a twice-daily regimen of sub-antimicrobial dose-doxycycline (SDD) or placebo tablets for two years as an adjunct to supportive periodontal therapy. Their results were consistent with a reduction in MMP-9 in the SDD arm.

In summary, there is limited evidence on the potential effect of periodontal therapy on MMPs levels suggesting a reduction of some of these biomarkers.

Oxidative stress

Free radicals are highly reactive species characterized by an unpaired electron in their outer orbital (Hall-

well 1991). They can damage (via oxidation) proteins, lipids, carbohydrates, and nucleic acids and ultimately contribute to a number of pathogenetic processes in a variety of inflammatory disorders. Reactive oxygen species (ROS) include oxygen-derived free radicals, such as superoxide, hydroxyl, nitric oxide, hydrogen peroxide, and hypochlorous acid (Waddington et al. 2000, Canakci et al. 2005). Oxidative stress is defined as the condition arising from a serious abundance of the levels of free radicals in a cell in comparison to its antioxidant defences (Singh & Jialal 2006). When antioxidant systems are unable to counteract the free radicals action efficiently, tissue damage ensues due to a number of processes including protein and nuclear oxidation, mitochondrial dysfunction ultimately leading to cell death (Sies 1997).

Oxidative stress has been implicated in a number of inflammatory diseases, such as type-2 diabetes (Evans et al. 2002), vascular diseases (Faraci 2005) and chronic inflammatory lung disease (Rahman & MacNee 1996). Oxidative stress does compromise endothelial cell function, a crucial mechanism in the development and progression of atherosclerosis (Bonomini et al. 2008).

In individuals suffering from periodontitis, excessive production of ROS has been demonstrated and appears as a result of local inflammatory responses (Chapple 1997, Takane et al. 2002, Guentsch et al. 2008, Su et al. 2009). Oxidative stress by-products including lipid peroxidation, protein carbonyl levels and antibodies against ox-LDL (Ox-LDL) are significantly elevated in individuals with periodontitis when compared to healthy controls (Akalin et al. 2007, Baltacioglu et al. 2008, Monteiro et al. 2009). Furthermore, a significant decrease in the antioxidant capacity of individuals suffering from periodontitis compared to healthy controls has been reported (D'Aiuto et al. 2010, Tamaki et al. 2011).

Eleven clinical intervention trials were found examining the effects of periodontal therapy on markers of oxidative stress (Appendix S9). Montebugnoli et al. (2005) in a clinical trial without control group described a significant decrease in

Ox-LDL in 18 males with confirmed CHD, 3 months after periodontal non-surgical therapy. In contrast, in a pilot study of 14 otherwise healthy subjects with severe periodontitis, a single session of intensive periodontal treatment triggered a substantial increase of oxidative stress as assessed by circulating ROS followed by a progressive reduction up to 1 month after therapy (D'Aiuto et al. 2010). These findings were confirmed by Tamaki et al. (2009) who showed that improvement in periodontal parameters 2 months after non-surgical periodontal therapy was associated with a significant reduction in plasma ROS level in 19 systemically healthy individuals suffering from periodontitis. In a second trial of the former authors, non-surgical periodontal treatment was also shown to result in a reduction in ox-LDL, and reduction of oxidative stress in 22 otherwise healthy subjects with chronic periodontitis (Tamaki et al. 2011). On the contrary, Lalla et al. (2007) and Koromantzos et al. (2012) both failed to report a reduction of measures of oxidative stress in individuals suffering from periodontitis and diabetes.

There is limited evidence on the effects of periodontal therapy on biomarkers of oxidation and it does suggest a possible reduction of these biomarkers in the medium term.

Discussion

After more than 30 years from the first reports on the association between dental infections/periodontitis and CVD outcomes, we are still debating on whether these associations are causal or casual in nature. Over the last 10 years, the number of clinical intervention trials investigating the effect of periodontal therapy on traditional and novel CVD risk factors and surrogate outcomes has increased. However, after a critical appraisal of the evidence reported to date, we confirm that there is still limited comparative evidence on the effect of periodontal therapy on CVD hard outcomes and or subclinical atherosclerosis.

Periodontal therapy, which primarily consists in the mechanical disruption of the dental biofilm of the diseased dentition, is often associ-

ated with a local and systemic amplification of the body inflammatory response. An acute inflammatory response (characterized by sharp increase in a number of inflammatory biomarkers, including CRP, IL-6, TNF- α) has been consistently reported by several investigators. This inflammatory state is also associated with a perturbation of the haemostatic system (fibrinogen, d-dimers, PAI-1) and a state of endothelial cell activation (sE-selectin, vWF) and impairment of endothelial function (as assessed by FMD of the brachial artery). The systemic implications of these acute effects are not fully understood at this time. Further investigations should be performed as to increase our understanding of the potential negative impact of periodontal therapy acutely in high risk populations including individuals with associated co-morbidities (i.e. diabetes mellitus or diagnosed CVD). There is already a wealth of evidence suggesting that episodes of acute infection/inflammation are associated with a short-term increased risk of vascular events including myocardial infarction and stroke (Smeeth et al. 2004, 2006, Schmidt et al. 2012) and one report suggests that this finding could also be true in individuals undergoing simple dental treatment procedures like a tooth extraction (Minassian et al. 2010).

Following 1–2 months from periodontal therapy, all comparative evidence retrieved by the authors is suggestive of a progressive reduction/improvement in traditional (lipid markers) and novel (CRP, IL-6, fibrinogen, sE-selectin) CVD risk factors. Interpreting this data with caution could represent the basis for inclusion of periodontal assessment and therapy as the key determinants in controlling each individual CVD risk. In particular, we found moderate evidence of a positive effect of periodontal therapy in reducing CRP levels and improving endothelial function within 6 months. Periodontal therapy, however, was not reported to reduce lipid fractions and IL-6 levels.

However, a number of flaws were identified when appraising the available evidence on the effect of periodontal therapy on CVD outcomes and should be highlighted. First, the

majority of clinical trials is of limited sample size (<500) reducing the precision and the variability of the estimates reported. Second, the length of follow-up of almost all trials is limited to 6 or maximum 12 months after therapy. Finally, we found only few multicentre studies. These findings represent perhaps the most important limitation in interpreting the current data on the effects of periodontal therapy on CVD outcomes. Both periodontitis and CVD represent long-term chronic conditions. In particular, atheroma formation has been identified very early in life (i.e. already in young children). We could speculate therefore that performing some periodontal treatment only at the end of atheroma evolution (much later in life) might not represent an effective method of preventing further progression of the disease nor the occurrence of acute vascular events. Further research efforts should be devoted in designing appropriate clinical trials on the delivery of effective oral health promotion early in life and monitor the potential beneficial effects of this approach on cardiovascular health. A number of promising biomarkers associated with future CVD risk (i.e. CRP, IL-8, MCP-1, sE-selectin) should be consistently investigated by researchers as to increase our understanding on the effects of periodontal therapy on atheroma progression and allow easier interpretation in the future of these findings with quantitative methods (meta-analysis).

Furthermore, the multifactorial aetiology of both periodontitis and CVD and the fact that both share a common inflammatory nature would indicate that the mere control/removal of local gingival infection might not be sufficient in producing a systemic sustained benefit. In turn, additional therapeutic approaches should be researched including host modulation therapies in combination with standard periodontal therapy. We report some limited evidence of combining periodontal therapy with SDD. These novel approaches at this time have not been tested also because of the limited knowledge of the mechanisms involved in the onset and progression of both periodontitis and CVD.

Conclusions

The main consistent finding after periodontal therapy was a reduction of serum levels of CRP (stable measure of systemic inflammation) and an improvement of measures of endothelial function (which represents a surrogate marker of CVD). Both biomarkers have been associated with increased future risk of CVD and therefore this would pose in favour of a potential beneficial effect of periodontal therapy in reducing CVD risk.

Despite being advocated as strong predictor of future CVD events, CRP serum levels, however, have recently being questioned whether being implicated in atherogenesis or merely represent a proxy measure of other unmeasured CVD risk factors (Casas et al. 2006). Nevertheless, we could speculate that the consistent reductions in CRP serum levels following periodontal therapy reported by several investigators pose in favour of a possible role of periodontitis in causing a state of systemic inflammation and potentially affecting a variety of chronic disorders including CVD and diabetes.

Although endothelial dysfunction is predictive of future CVD risk/outcomes, it is also a research measure greatly confounded by a number of methodological and environmental factors and therefore does not represent an efficient research outcome to be implemented in large scale intervention trials. Surrogate measures of sub-clinical atherosclerosis including c-IMT have been extensively adopted in CVD intervention trials and consistently show a strong association with future risk of CVD events including myocardial infarction and stroke. No evidence was found on the effect of periodontal therapy on c-IMT and other measures of subclinical atherosclerosis. Authors would therefore suggest using a surrogate marker of CVD like c-IMT in future trials attempting to demonstrate a positive effect of periodontal therapy on CVD. In addition, large scale/multinational intervention trials have not been conducted on the effect of periodontal therapy on traditional or novel CVD risk factors. Further and more importantly, most of the clinical tri-

als found by the authors in this review were performed in university/hospital settings which perhaps would not well represent the everyday periodontal clinical care. Ultimately, large multi-centre clinical trials assessing the effect of periodontal therapy on the incidence of future CVD events are needed to confirm or dispute the causal association between periodontitis and CVD.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1. Effects of periodontal therapy on lipids.

Appendix S2. Effects of periodontal therapy on blood pressure.

Appendix S3. Effects of periodontal therapy on endothelial function.

Appendix S4. Effects of periodontal therapy on white cell counts.

Appendix S5. Effects of periodontal therapy on acute phase reactants.

Appendix S6. Effects of periodontal therapy on interleukins/TNF- α , CD40/MCP-1, and soluble adhesion molecules.

Appendix S7. Effects of periodontal therapy on haemostatic factors.

Appendix S8. Effects of periodontal therapy on MMPs.

Appendix S9. Effects of periodontal therapy on oxidative stress.

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Clinical Relevance

Scientific rationale for the study: Periodontitis and cardiovascular diseases are common chronic disorders of the general population. Although a large number of observational studies support an association between periodontitis and cardiovascular disease, over the last two decades a causal link between these disorders has been repeatedly proposed but never proven. Indeed,

these claims have produced lot of uncertainty in the dental and medical community around the potential benefit of controlling periodontitis upon the progression of vascular diseases and/or reducing the risk of future CVD events.

Principal findings: Periodontal interventions produce acute and chronic effects including impairment first and improvement later of endothelial function and inflammatory burden.

There is however limited evidence on the effect of periodontal therapy on cardiovascular mortality.

Practical implications: There is moderate evidence suggesting that periodontal therapy reduces systemic inflammation and improves endothelial function, but limited evidence on its effects on cardiovascular events in the long term.