Reviewing the Mechanisms and Causal Relationship Between Periodontitis and Cardiovascular Disease

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As cardiovascular diseases continue to prevail as the leading cause of death worldwide, identifying risk factors that increase risk of CVD is more necessary than ever. Periodontitis has been suggested as a risk factor contributing to the development of CVD in the early 20th century; however, the evidence behind it remains controversial to this day and no clear consensus has been reached. In this review, I focus on the most plausible and well researched mechanisms that have been suggested to play part in bringing about CVD as a consequence of periodontitis. Seemingly, there is convincing evidence behind all the mechanisms discussed, although, none have been undoubtably proven. The evidence does suggest a causal relationship between the two, with periodontal treatment improving CVD-related risk factors; however, the evidence remains insufficient. The reason for this could lie in the fact that cohorts of this nature bring about ethical implications and sample sizes haven't yet been sufficient to draw definitive conclusions. The studies discussed in this review suggest both a causal and a non-causal relationship and I highlight their most prominent features as well as identify their shortcomings. I present a non-biased overview of the available evidence, and equally give emphasis on both the evidence suggesting a link between CVD and periodontitis as well as the evidence claiming no link between the two.

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide and encompass a group of disorders of the heart and blood vessels. CVD includes amongst others, coronary atherosclerosis, cerebrovascular disease and peripheral vascular disease. An estimated 17.9 million people died from CVDs in 2019, representing 32% of deaths worldwide. Globally, prevalence is rising with an ageing population and lack of regular physical activity becoming the norm. Traditional risk factors of CVD include hypertension, smoking, abdominal obesity, and stress.[1] Manifestations of CVD mostly occur in people with multiple modest risk factors which are often unnoticed.[2] It is therefore important to treat or tackle any risk factors and intervene as early as possible, for example through screening the healthy population.[3]

Inflammation and infection have been shown to play a role in CVD, with atherosclerosis, the primary cause of stroke and coronary artery disease, being regarded as a chronic inflammatory disease. Atherosclerosis involves a build-up of lesions and plaques inside the artery and is largely driven by an innate immune response against the vessel wall involving monocytes, macrophages and pro-inflammatory cytokines.[4,5] Autoreactive helper T-cells can also be found in atherosclerotic plaques, and are specific to Apolipoprotein B, which has the function of trafficking lipids around the body.

Periodontal disease encompasses diseases affecting the gingiva, the supporting connective tissue and the alveolar bone.[6] It is separated into diseases that only involve the gingiva, a.k.a. gingivitis, and the more troubling periodontitis, which is involved the destruction of the periodontium.[7] The disease entails progressive destruction of tissues supporting the teeth, such as the periodontal ligament, the gingival and the alveolar bone.[8,9] It was the sixth most prevalent health condition worldwide in 2010, affecting 743 million people.[10] Gingivitis disease is caused by oral microbes, including actinomyces species and streptococcus.[11,12] Periodontitis is

mainly caused by a limited number of pathogenic microbes in the biofilm that forms in the infected environment, and are mostly anaerobic.[13–15] There are however many non-pathogenic microbes that inhabit the oral microbiome which can contribute to the formation of periodontitis, when interacting with the micro niche, such as Veillonella *parvula*.[16] Periodontal bacteria are found in the crevicular fluid, and damage caused by the disease is both due to products released by the bacteria in the plaque, and the host immune response.[17] Products such as H_2S, amines, collagenases etc. released by the bacteria illicit an immune response, leading to a net loss of periodontal tissue.[18] Periodontitis is associated to an increase in levels of systemic inflammation markers, such as FGF-21, HGF, and interleukin-18R1 and show an increase in blood leukocyte levels.[19,20] The severity of periodontal disease is also associated with C-reactive protein levels (CRP), which is also associated with risk of CVD.[21–23] The disease occurs amongst all age groups, but mostly in the elderly due to prolonged exposure to risk factors.[8,9] These risk factors include smoking, diabetes mellitus, and stress, which are also risk factors for CVD. Periodontitis has been linked as a risk factor for many diseases including diabetes, arthritis and osteoperosis. It is also theorised to cause cardiovascular disease.[24]

The mouth has been a known source of bacterial infection since the early 1900s.[25,26] This was mostly in relation to dental extraction, where streptococcal bacteraemia was detected after a procedure. Following these findings, the American Heart Association recommended antibiotic treatment prior to dental extraction for patients suffering from rheumatic heart disease and coronary heart disease, as bacteria can lodge onto the heart valves or other parts of the endocardium, causing endocarditis.[27] In 1989, a Finnish research group reported that patients with an oral infection were 30% more likely to have a myocardial infraction, and that the same patients had a higher degree of artherosclerosis the worse their infection was.[28] The data was also adjusted for other known risk factors, such as smoking, cholesterol levels, and HDL, and was one of the first associations reported between oral infection and heart disease. Further cohort studies during the 1990s showed that people with periodontitis were 25%, and 1.5 times more likely to develop coronary heart disease, when adjusted for other risk factors.[29,30]

Even though there seems to be cooccurrence between the two diseases, it is a controversial matter whether CVD and periodontitis have a causal relationship. After years of evidence linking CVD with periodontitis the American Heart Association released a report stating that there is not enough evidence to support the theory that periodontitis can cause CVD. Treatment of periodontitis was unable to prevent or change the outcome of atherosclerotic vascular disease, showing that the link could be due to confounding factors.[31] Collider bias has also been proposed as a reason for mistaking causality of periodontitis, as levels of inflammatory markers distorting the association between the two diseases.[32] In this review I will discuss the potential links between periodontitis and cardiovascular disease, with attention to controversies regarding the causality of periodontitis for CVD.

Proposed Mechanisms for Causality Between CVD and Periodontitis

General Susceptibility Model:

In this model there is no causal link between CVD and periodontitis, but a common genetic locus that makes the person susceptible to both CVD and periodontitis. The study was done using 1,104 individuals with severe coronary heart disease (CHD) and the subject population was composed entirely of German individuals. Genomic DNA was obtained from their blood samples and amplified by whole genome amplification and subsequently genotyped. Consequently, Schäfer et al. described in their paper a susceptibility locus on chromosome 9p21 which is involved in ANRIL activity and associated with CHD and aggressive periodontitis.[34] ANRIL is an antisense lncRNA and presents a variety of different isoforms. Polymorphisms at the ANRIL gene have been linked with a plethora of metabolic diseases

amongst which is CHD.[35] The mechanism behind how exactly ANRIL polymorphisms bring about CHD is not clearly understood as most ANRIL exons do not exist in mice, however it is thought to modulate the immune response through interaction with the Yin Yang 1 protein.[35] It has a protective role against periodontitis, and downregulation of ANRIL is associated with higher CRP levels in sera, which is also a marker for CHD.[36,37] In conclusion, an individual would be at a greater risk of CVD, and upon a bacterial infection have a higher risk of developing periodontitis. This would therefore create an association between the two, as these people are more likely than the average population to display both CVD and periodontitis. The two diseases also share many risk factors, such as smoking, diabetes mellitus, obesity, metabolic syndrome, meaning that if one were to have any of the risk factors, a comorbidity of periodontal disease and CVD would be more likely, leading to an association of the two diseases.

The presence of such a susceptibility locus does however not remove the possibility of other mechanisms underlying the correlation between CVD and periodontitis, with the general susceptibility model partly explaining the link between the two. It does however not rule out other mechanisms explaining the link between the two diseases.

Systemic Inflammation model:

Systemic inflammation is a risk factor for cardiovascular disease.[38] Systemic inflammation involves higher levels of circulating cytokines and inflammatory mediators. One marker for systemic inflammation is CRP and having a higher base-line plasma concentration of CRP is predictive of future myocardial infraction and stroke.[39] Treatment of inflammation with aspirin for example is able to reduce the level of CRP and the risk of myocardial infraction.[39] Complement factors and cytokines such as CRP, IL-1, IL-6 etc. damage the vascular endothelium and can lead to atherosclerosis for example.[40]

Periodontitis has also been found to increase blood levels of CRP and interleukin-6, which in turn leads to increased levels of endothelial activation markers E-selectin and von Willebrand factor in sera.[41] The increase in inflammation markers could be due to spill over of inflammatory mediators from the local inflammation at the site of the infection into the blood stream, and this model does not involve any bacteraemia. In the study by Tonetti et al.,

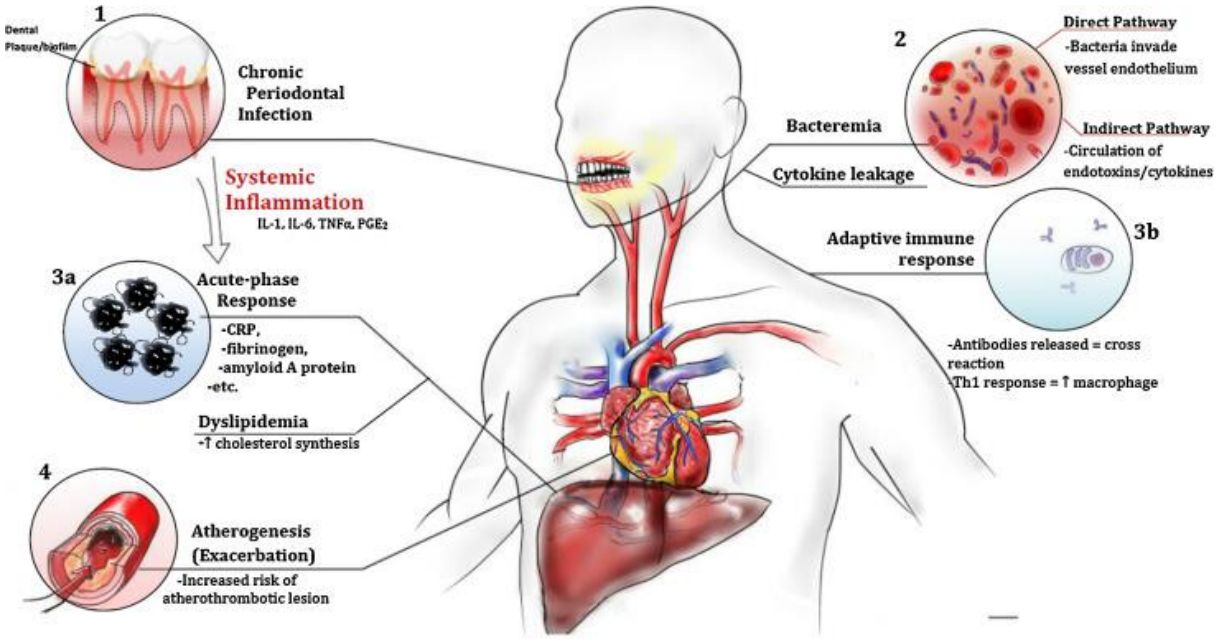

Fig. 1: Mechanisms for causality between CVD and periodontitis. Shown are the systemic inflammation response caused by periodontitis (1), the infection model (2), and the molecular mimicry model (3b).

120 patients were randomly assigned to community-based care involving supragingival mechanical scaling and polishing, whilst the intensive treatment group underwent a cycle of adjunctive full-mouth intensive removal of subgingival dental plaque biofilms with the use of scaling and root planning. In addition to this they received the antibiotic minocycline in the periodontal pockets. Endothelial dysfunction present in the patients was reduced in the group undergoing intensive treatment compared to the community-based care group, showing a clear link between periodontitis and endothelial dysfunction.[41] Other studies have also shown an increase in CRP associated with chronic periodontitis, and that the highest levels of CRP are seen in more advanced periodontitis.[42,43] In this model there is therefore a causal link between CVD and periodontitis, with periodontitis increasing inflammatory markers and cytokines, leading to endothelial dysfunction and CVD.

A limitation of this model would be if a collider bias is present in the link between the two diseases. CRP is often used as the common marker for periodontitis and CVD, but this association could be false if CRP is caused both by periodontitis and heart disease, as will be explained later in the review. Moreover the use of surrogate endpoints, such as CRP and IL, is not always associated with benefits to clinical endpoints, such as lower cardiovascular disease levels. It is attractive to do a study on surrogate endpoints because it does not require such a long study span to see effects on patients.[44] Just because a surrogate is impacted by an intervention, it does however not mean that it is part of the causality of the link between CVD and periodontitis. Further studies on clinical endpoints associated with CVD would need to be carried out and specific pathways would need to be determined to solidify this model.

Infection model:

Contrary to the systemic inflammation model, here the periodontal bacteria infect the blood stream directly and invade the endothelium, later leading to CVD due to endothelial dysfunction and inflammation. The bacteria do not stay in the mouth and cause more direct harm to the endothelial system systematically.

In support with the infection model, several bacteria associated with periodontitis have been found in atherosclerotic plaques and patients with coronary artery disease.[45,46] It has also been reported that dental probing of periodontitis and people with periodontitis brushing their teeth can cause bacteraemia and lead to endocarditis.[47,48] The periodontopathic bacteria P. *gingivalis*, Fusobacterium *nucleatum*, and Tannerella *forsythia* were found in artery specimens. Bacteraemia caused by oral pathogens can occur following toothbrushing, or periodontal probing for example, as these can cause oral bleeding, upon which bacteria enter the bloodstream.[48,49] The presence of these bacteria in the plaques however does not mean that they are the cause of them, or that they make them any worse. It merely associates the two and could indicate a link between them, or it could merely be an opportunistic infection of an already damaged tissue.

Molecular Mimicry Model:

The molecular mimicry model hypothesises that heat-shock proteins (HSPs) released by bacteria are so similar to human heat-shock proteins (hHSPs), that it could illicit an autoimmune response, leading to cardiovascular disease.[50] Cells express HSPs upon being stressed by heat, which is elevated in an immune response. Bacteria as well as humans express HSPs in response to stress, and the proteins are similar in sequence, with a 50% homology between mycobacterial HSP65 and human HSP60.[51] Upon infection of the body by bacteria, including periodontal bacteria, an adaptive immune response against the infection would be mounted, and antibodies against bacterial HSP could be formed. When human endothelial cells are mistreated, for example by smoking, obesity, or infections, then they too express hHSP60. Due to homology of the sequences, the antibodies bind to the hHSP and could lead to atherosclerosis.[50,52] This is then followed by the development of more severe lesions and plaque formation. It has been found that anti-HSP60 antibodies are cross-reactive with those of other bacteria, and were able to lyse stressed endothelial cells, and that periodontitis patients show antibodies to gingivalis GroEL, and cross-reactivity to hHSP60.[53,54] There is also a correlation between high anti-HSP60/65

antibody levels and high mortality due to atherosclerosis.[55] Presence of other risk factors for CVD, such as high cholesterol, also enhances hHSP expression.[50]

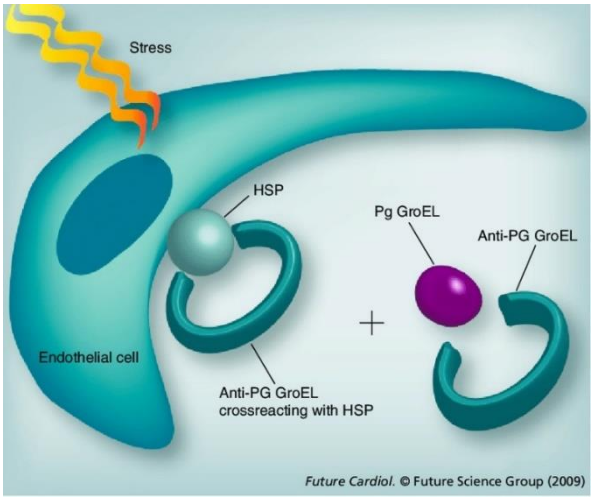

Fig. 2

Mechanism of molecular mimicry. Shown are anti-P. *gingivalis* antibodies crossreacting with HSP expressed by a stressed endothelial cell.

It was found that in patients with CVD, a more severe periodontitis characterized by increased levels of P. *gingivalis* and T. *forsythia* is associated with higher levels of anti-hHSP60, meaning severe periodontitis is associated with higher autoimmunity.[56] Infection of ApoE deficient mice with P. *gingivalis* also worsens atherosclerosis and lesion size correlated with anti-GroEL antibodies, showing that the association holds up in an animal model as well.[57] Immunization of ApoE deficient mice with anti-hHSP60 antibodies also increases severity of atherosclerosis.[58]

All together evidence does show that molecular mimicry could be a mechanism underlying the link between the two diseases, especially via the support of the animal model. Further proof would however be needed to make the mechanism concrete, such as immunization of mice with anti-GroEL antibodies and subsequent observation of atherosclerosis severity.

Evidence for Periodontitis as a Risk Factor for Cardiovascular Disease

Link between Periodontitis and Cardiovascular Disease

It is well established that an association exists between CVD and periodontitis, with CVD being the most common systemic condition in periodontitis patients.[59] A recent meta-analysis including thirty longitudinal cohort studies including clinically and self-reported measurements showed that the risk of CVD was higher in periodontitis patients compared to non-periodontitis patients (relative risk: 1.2).[60] Severe periodontitis also increased the risk for CVD, with stroke and the CHD risk being the highest. Especially the increased risk of CVD in severe-periodontitis patients points to periodontitis possibly being causative for CVD.[60] Periodontitis has been linked to many forms of CVD, such as hypertension, stroke, atherosclerosis, and coronary heart disease, showing that periodontitis could lead to CVD through a number of ways.[61–64] Finally, in 2009 a meta-analytical study found that the risk of developing CVD was 34% higher in patients with periodontitis.[65]

Positive Studies

A possible proof for periodontitis being causative of CVD would be if treatment of periodontitis reduced the risk or severity of CVD. Treatment of periodontitis can be surgical, including flap surgery, tissue or bone grafts, or nonsurgical, including rooting and planning or antibiotics.[66] Studies from 2019 until the 26th of June 2022 were selected from PubMed under the search term “periodontitis and cardiovascular disease”, including randomized control trials and clinical trials. Periodontal treatment could also have a net negative effect on cardiovascular health, as treatment can cause bacteraemia and inflammation. In a randomized controlled trail including 48 periodontitis patients with a recent myocardial infraction, the flow-mediated vasodilation (FMD) in the brachial artery was tested with and without treatment to assess

endothelial function 6 months after treatment, with a higher percentage of FMD indicating improved endothelial function. Significant flow-mediated vasodilation improvement was seen in the group undergoing periodontitis treatment, (from 9.0% ± 4.4% at baseline to 12.1% ± 5.6% at follow-up; p = .01), but not in the control group (from 12.2% ± 7.2% at baseline to 11.9% ± 4.0% at follow-up; p = .79), indicating that treatment improves endothelial function in the patients.[67] The low number of patients and the fact that it was done in one clinic in Brazil does however reduce the statistical power of the study. Improvements in serum cytokines were also not seen in the patients undergoing treatment.

Another study looked into the effects of periodontal treatment on metabolic syndrome, a major cause of cardiovascular disease. 112 patients with metabolic disease received either a dental intervention along with dietary and exercise guidance, or just dietary and exercise guidance with no dental intervention. They were tracked for 3 months after their intervention. In the end the dental intervention did improve their BMI, fasting blood sugar level, and anthropometric status and reduce the risk of metabolic syndrome.[68] This again indicates that the dental intervention improves risk factors for cardiovascular disease. With the assessment of the patients only being 3 months there is however a limitation on the long-term effects of the treatment. Authors of the paper stated that due to ethical implications, treatment of periodontitis could not be forgone for longer than 3 months. The trial was also voluntary, meaning that mostly motivated participants looking to improve their health were included, which could have a confounding effect on the outcomes.

Another study in Brazil showed that in patients with a recent ST-segment elevation myocardial infraction, the most acute form of coronary artery disease, periodontal treatment also significantly improved endothelial function.[69] 48 patients were either part of the control, or treatment, group, receiving treatment or not. After 6 months cytokine levels and FMD measurements were taken, with both improving in the treatment group. Again, a low number of patients originating from one clinic diminishes the power of the study.

A larger study involving 206 patients on a waitlist for haemodialysis tested the effects of periodontal treatment on the 24-month incidence of cardiovascular events and cardiovascular death.[70] The main findings included that coronary artery disease correlated with the severity of periodontal disease. The control group contained 203 historic patients who did not undergo periodontitis treatment. Multivariate analysis showed that there was a reduction in cardiovascular events (HR 0.43; 95% CI), coronary events (HR 0.31; 95% CI), and cardiovascular deaths (HR 0.43; 95% CI) among the treatment group. The use of historic patients in this trial allowed for a longer follow up period, as ethical implications of forgoing treatment on patients suffering from periodontitis does not play a role, and the large patient population strengthens the results. The authors themselves however do state that the population is still relatively small, and there was no randomization of the patients. The fact that historic controls were used also means that no cause-effect relationship can be drawn from the study.

An older study by Rastogi et. al. looked at levels of inflammatory markers associated with CVD before after periodontitis treatment.[71] 20 patients underwent non-surgical periodontal therapy including root scaling and planning and oral health advice and markers were analysed prior to and 1 month after periodontal therapy. It was found that 1 month after periodontal therapy hsCRP, and white blood cell counts were reduced significantly, however there was not a reduction in tumour necrosis factor alpha. Despite the low patient count, the fact that reduction of hsCRP and white blood cell levels was significant in all patients does support the idea that periodontal therapy can decrease inflammation long term, through which CVD could possibly be prevented.

Together these articles indicate an improvement in endothelial health and a reduction in risk factors for cardiovascular disease after periodontal treatment, which could indicate that treatment could be administered as a preventative measure for cardiovascular

disease. There are however limitations to the studies, as will be discussed later in the review.

Negative Studies

Contrary to the aforementioned treatment studies, there are studies showing that periodontal treatment had no significant effect on cardiovascular risk factors. A study including 90 patients with peripheral arterial disease and severe periodontitis were included in the trial, with 30 undergoing periodontal treatment and antibiotics, 30 undergoing periodontal treatment without antibiotics, and 30 undergoing no treatment. Patients were observed 3 months after treatment.[72] Whilst periodontal health did increase in the treatment groups, no difference in the aorta was observed (median target to background ratio follow-up/baseline, PT1 1.00; 95% CI 0.97–1.10, PT2 1.00; 95% CI 0.98–1.1, CG 1.1; 95% CI 0.99–1.1, $p = 0.75$). There were also no differences in vascular biomarkers between groups, including oxLDL, angiopoetin-like protein 4, serum myeloperoxidase, free fatty acids, and endothelial thrombin potential. Again, a low number of patients reduces impact of the study. Only atherosclerosis patients with symptomatic claudication were recruited, and the authors state that more severe forms of atherosclerosis, such as critical limb ischaemia may show different results.

A further randomized controlled trial involving 110 patients followed vascular function markers FMD and serum asymmetric dimethylarginine (ADMA) levels in periodontitis patients for 3 months.[73] Patients in the control group received standard treatment, whereas the advanced treatment group received additionally applied disinfectant to their gums. Whilst the test group showed significant improvements in their periodontitis compared to the control group, there was no improvement in FMD and ADMA levels. This therefore does not indicate a link between improvement in periodontal care and improvement in cardiovascular health. The fact that follow up was only after 3 months limits the impact of the study, and they also relied on self-reports from the patients regarding the advanced self-care treatment.

As mentioned earlier, Collider bias has been proposed as a reason for a false association between periodontitis and CVD. A 2019 article investigated the effects of high-sensitivity C-reactive protein (hsCRP) as a confounding factor for the association between the two diseases.[32] A collider bias exists when the exposure and the outcome of a scenario cause a third, independent variable, and when a study controls for this variable, it introduces an association where there is none. In this case, periodontitis is the exposure and CVD the outcome. The article proposes hsCRP as the collider, with periodontitis increasing levels of CRP, along with a genetic condition (APOE-CI-CII), which is associated with both increased hsCRP, and CVD. As hsCRP is therefore higher in periodontitis and CVD patients and is often used as a biomarker to measure the association and is proposed as a link in the cause-effect relationship, it may be a confounding variable. Data from 480 members of the 1982 Pelotas Cohort was used to test this effect. When controlling for high hs-CRP levels, Febbraio et al., failed to show a correlation between CVD and periodontitis; indicating that there is no direct link between the two conditions. That is, when hsCRP levels are not high no clear link between CVD and periodontitis is present. However, when employing individuals with higher hsCRP levels, they found a very strong association between the two. The data from this study therefore suggests that elevated hsCRP levels create a biased and inaccurate association between periodontitis and CVD. The fact that when controlling for hs-CRP levels, the association disappears, does however not rule out the fact that hs-CRP could play a biological role in the link between the two, and the study could not rule out residual confounding factors to be accountable for high hsCRP levels, such as smoking. More studies including hsCRP and other biomarkers are therefore required to further investigate the role Collider bias plays in the relationship.

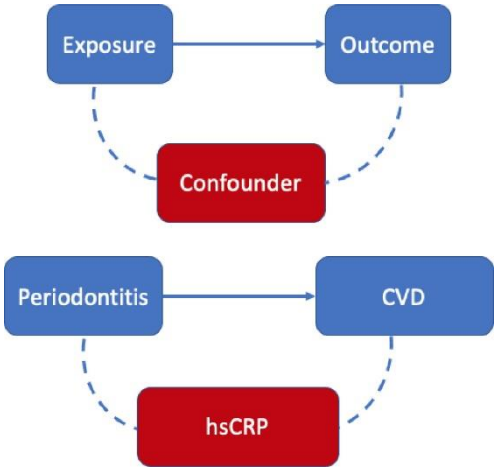

Fig. 3

Representation of the collider bias, with periodontitis as the exposure, CVD as the outcome, and hsCRP as the confounder/collider.

Negative Effects of Periodontal Treatment

As previously mentioned, periodontal treatment can cause bacteraemia and lead to carditis.[27] It also causes inflammation, a risk factor for CVD. If periodontitis treatment therefore did not reduce the risk of CVD via reducing periodontal disease, it could overall cause an increase in risk of CVD via periodontal treatment.

Platelet activation is a risk factor for thrombosis and atherosclerosis, and is increased in patients with periodontitis.[75–77] A 2019 therapeutic trial included 26 patients who underwent periodontal treatment, with their blood being analysed immediately before and after treatment for platelet activation markers, such as P-selectin, CD63 and CD40L.[78] No significant changes in platelet activation levels were observed before and after treatment, and there was no difference in platelet reactivity to varying concentrations of the platelet agonist ADP. This therefore indicates that periodontal treatment does not pose a threat to short-term platelet activation related illnesses. The trial did however include a very small number of patients, and no control group. As they only observed platelet activation immediately after treatment, no conclusions can be drawn about platelet activation at later time points.

Another study by Morozumi et. al. analysed inflammatory mediators 1 day after a full-mouth scaling and root planning.[79] 31 severe or chronic periotontitis patients received the treatment, and were followed up on 1 day and 6 weeks after the treatment. 1 day after treatment, patients body temperature, CRP, interferon gamma, and interleukin-12p70 increased in the serum samples. 6 weeks after treatment, a significant decrease in bacterial count and IgG titers against P. *gingivalis* was found. Again a limitation in the study is the sample size, however this study does show that there is an increase in inflammation markers after periodontitis treatment at first, however after 6 weeks, bacterial counts decrease along with antibody levels against P. *gingivalis*, indicating a decrease in infection. Along with a previously shown decrease in inflammatory markers 1 month post-treatment of periodontitis, this would indicate an overall improvement in risk-factors for CVD.[71]

Overall it can therefore be said that in spite of the short term elevation in inflammatory markers, the long term benefits of reducing inflammation outweigh this effect, reducing the overall presence of risk factors for CVD.

Discussion

Periodontitis has been associated with cardiovascular disease for many years, with controversies over the cause effect relationship between the two diseases. Whilst patients with severe periodontitis are more likely to develop CVD, even when adjusted for common risk factors shared between the diseases, the exact mechanism as to which periodontitis could cause CVD has yet to be set in stone. There are four mechanisms proposed, with the general susceptibility model stating a common genetic susceptibility to CVD and periodontitis and forgoing a cause-effect relationship, the systemic inflammation model involves the periodontal infection causing an increase in inflammatory markers leading to CVD and the infection model involves direct infection of the periodontal pathogens into the blood stream,

causing e.g. worsening of atherosclerosis. Finally, the molecular mimicry model proposes that GroEL released by periodontal pathogens is homologous to hHSP60 and anti-GroEL antibodies are cross-reactive to hHSP60, causing an autoimmune response.

There is convincing evidence for each of the theories, and it must be said that one mechanism being causative of the association does not rule out other mechanisms playing a role too. There are however weaknesses in some of the mechanisms and their evidence. Alongside ANRIL there have been multiple other shared susceptibility loci for periodontitis and cardiovascular disease.[80,81] The existence of the loci has been determined via genome analysis, however further studies on the exact mechanisms of disease caused by the genetic variation still need to be carried out to confirm the loci as a shared genetic susceptibility and confirm that the loci play a role in the association between CVD and periodontitis. The main pitfall of the systemic inflammation model is the use of surrogate endpoints and lack of trial time and sample size. This is a pitfall of all periodontal studies, as it is ethically not possible to forgo treatment of periodontitis for longer than 3 months. The evidence for this theory is therefore lacking, however the fact that periodontitis does cause inflammation, and the fact that inflammation is a risk factor for cardiovascular disease, should make the model hold up. Longer studies with a higher sample size need to be carried out, and the Collider effect needs to be taken into account, which current studies fail to do.

The infection and molecular mimicry models share similar evidence in the form of the mouse model. The fact that intraperionatal infection of mice with P. gingivalis worsens atherosclerotic plaques claims to prove the molecular mimicry theory, however it only proves the infection model, with bacteraemia causing CVD. Molecular mimicry studies so far fail to show that GroEL antibodies can directly cause CVD, with studies only showing their cross-reactivity to hHSP60, but their effect in an animal model has yet to be shown.

With this evidence taken together it is likely that all mechanisms could play a role in the association between CVD and periodontitis, however more evidence is specifically needed for the genetic susceptibility, systemic inflammation, and molecular mimicry models.

The same problems that are faced in showing the systemic inflammation mechanism are also present in the trials showing whether treatment improves a periodontal patient's chances of developing CVD. The studies all show low sample size, and the study with the highest has historical controls, not allowing for a cause-effect relationship to be deduced. The use of surrogate endpoints such as CRP also does not allow for a definitive answer on whether CVD could be prevented via periodontal treatment, and it does not allow for the elucidation of the mechanism via which periodontitis could cause CVD. A study set up which could be beneficial here is having a control group which undergoes basic periodontal treatment and comparing them to a group which undergoes more advanced treatment, such as in the trial by Tonetti et al.[41] This could allow for a longer follow up over years and a focus on clinical endpoints, with a higher sample size.

The evidence against periodontal treatment having a positive effect on CVD is also diminished heavily by low sample sizes, the use of surrogate endpoints, and short follow up time. A larger cohort study is therefore needed to end the debate, however the evidence does point towards periodontitis having a causal relationship with cardiovascular disease, even if it maybe smaller than the current evidence suggests.

The general susceptibility model stating a common genetic susceptibility to CVD and periodontitis could explain part of the relationship but does forgo a cause-effect relationship. The bacteria infecting the periodontal tissue causing periodontitis cause inflammation and can infect the bloodstream and have been found in atherosclerosis plaques for example. It has been long accepted that inflammation is a risk factor for cardiovascular disease, and periodontitis does increase presence of inflammatory markers.[41,82] A cause-effect relationship based on this association may however be misleading, due to

the collider effect, where both periodontitis and a general susceptibility to high CVD and CRP levels cause high CRP levels, meaning that controlling for CRP will introduce a bias in the results. The studies mentioned used CRP as an inflammatory marker, however when Leite et al. separated periodontitis patients based on CRP levels, it was found that there was only a correlation between CVD and periodontitis in patients with high blood CRP levels, supporting the case for there being a collider bias in the association.[83] Additionally, the mere presence of periodontal pathogens in atherosclerosis plaques does not mean that they are caused by the pathogens, however intraperionatal infection of mice with P. *gingivalis* does worsen atherosclerosis symptoms, showing that the model holds up in an animal model.[57] Molecular mimicry is the final method proposed, as GroEL released by P. *gingivalis* and other periodontal pathogens has homology with hHSP60.

Together these mechanisms are not entirely proven. The use of surrogate endpoints means that the exact cause effect relationship of inflammation caused by periodontitis cannot be elucidated. In addition to this the short period of follow up does not allow for significant results regarding clinical endpoints, which is a side-effect of these trials, as periodontal treatment cannot be forgone for too long, otherwise it would present a risk to the patient. The use of more advanced periodontal treatment against a more standard treatment group seems more attractive here, as a longer follow up and larger sample size could be used, however ethical implications of not giving patients optimal treatment needs to be considered.

Whilst the mouse model provides support for both the infection and molecular mimicry model, more proof is required regarding the molecular mimicry model as to the effect of anti-GroEL antibodies against periodontal pathogens GroEL on atherosclerosis in the mouse model, as this would show that the antibodies formed in the clearance of a periodontal infection could impact atherosclerotic plaques and lesions and directly cause CVD.

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