Serum antibody response against periodontal bacteria and risk of coronary heart disease: systematic review and meta-analysis

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Running title: Periodontal bacterial antibodies and CHD risk

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Acknowledgements: The authors would like to thank Dr Neil Scott from the Medical Statistics Team, Division of Applied Health Sciences, the University of Aberdeen for providing statistical support and advice in conducting meta-analyses.

Disclosures and funding note: There are no financial disclosures or conflicts of interest to declare for any of the authors. This project received no external funding.

Word count: 4800 (ecluding abstract, clinical relevance, references, tables and figures)

Abstract

Background: The present systematic review and meta-analysis assessed the strength of a reported association between elevated serum anti-periodontal bacterial antibody responses and an increased risk of coronary heart disease (CHD).

Review: Twenty original studies were identified after systematically searching five databases. The majority (n=11) compared serum anti-*Porphyromonas gingivalis* (Pg) and/or anti-*Aggregatibacter actinomycetemcomitans* (Aa) IgG antibody responses between CHD patients and control participants. The strength of the association between serum anti-Pg antibodies and CHD (n=10) and serum anti-Aa antibodies and CHD (n=6) was investigated using a meta-analysis approach separately.

Results: Most studies (61%) reported that the serum IgG antibody responses were elevated in CHD patients than in controls. The meta-analyses showed a significant association between elevated serum IgG antibody responses (anti-Pg and anti-Aa) and CHD risk, with pooled odds ratios of 1.23 (95% CI: 1.09-1.38, p=0.001) and 1.25 (95% CI: 1.04-1.47, p=0.0004), respectively.

Conclusion: A modest increase of CHD risk in individuals with higher serum anti-Pg and anti-Aa IgG antibody responses may support their use as potential biomarkers to detect and monitor at-risk populations. However, observed inconsistencies with the design and interpretation of immunoassays warrants standardisation of the immunoassays assessing antibody responses against periodontal bacteria.

Systematic Review Registration: PROSPERO Registration Number: CRD42017082259

Keywords: Myocardial Infarction, Periodontitis, Atherosclerosis, Coronary Artery Disease, Antibodies, Immunoglobulin G, *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans*

Clinical Relevance

Scientific Rationale

An association between serum antibodies against periodontal bacteria and CHD is suggested but a consensus is lacking.

Principal findings

Elevated serum anti-*P. gingivalis* and anti-*A. actinomycetemcomitans* IgG antibody responses were consistently associated with CHD. However, given the inconsistencies in ELISA methodologies and the cut-off to differentiate high vs low antibody responses amongst included studies, assay standardisation using large-scale studies is warranted.

Practical implications

The ELISA technique to detect serum anti-*P. gingivalis* and anti-*A. actinomycetemcomitans* IgG levels can be utilised to screen and identify people at risk of CHD, and potentially find a clinical application to monitor future CHD risk.

Introduction

Periodontitis is characterised by chronic inflammation of the tooth-supporting structures, caused by dysbiotic subgingival microbiota and worsened by risk factors, such as smoking and uncontrolled diabetes. An accumulating body of evidence has shown that untreated periodontitis is associated with several systemic conditions such as diabetes, rheumatoid arthritis, adverse pregnancy outcomes, renal diseases and coronary heart disease (CHD), including myocardial infarction and angina pectoris (Deschamps-Lenhardt, Martin-Cabezas, Hannedouche, & Huck, 2019; Joshi et al., 2019; Nguyen, Nguyen, Huynh, Le, & Hoang, 2020; Olsen, 2021; Potempa, Mydel, & Koziel, 2017; Preshaw & Bissett, 2019).

The association between periodontitis and CHD is underscored by the recent consensus reports and meta-analyses, but a mechanistic link between the two conditions is yet to be established (Sanz et al., 2020; Shi et al., 2016; Tonetti, Van Dyke, & Working group 1 of the joint EFP/AAP workshop, 2013; Xu et al., 2017). One of the suggested links is the cross-reactivity between circulating antibodies against major periodontal bacterial species i.e. *Porphyromonas gingivalis* (Pg), *Aggregatibacter actinomycetemcomitans* (Aa) and several non-bacterial self-antigens, such as endothelial heat shock protein-60 (HSP-60), cardiolipin, malondialdehyde-modified, malondialdehyde acetaldehyde-modified and copper-oxidized low-density lipoprotein (MDA-LDL, MAA-LDL, Cu-oxLDL, respectively) (Sanz et al., 2020; Schenkein & Loos, 2013) (***Fig. 1***). It is hypothesised that the cross-reactive antibodies elicit pro-inflammatory responses that drive coronary atherosclerotic plaque instability, increasing the coronary artery disease risk (Akhi et al., 2017; Tabas & Lichtman, 2017; Wolf & Ley, 2019). The antigen-antibody complexes, particularly IgG antibodies bind to Fc receptors (FcγRI, FcγRIII and FcγRIV) on the effector immune cells, such as macrophages and natural killer cells, leading to antibody-dependent cellular cytotoxicity (ADCC) and Th1 pro-inflammatory responses. These complexes also activate complement-dependent cytotoxicity (CDC) (Tabas & Lichtman, 2017; Tsiantoulas, Diehl, Witztum, & Binder, 2014). These mechanisms are potential contributors to atherosclerotic plaque instability and rupture.

This hypothesis is mostly based on the studies reporting that sera of CHD patients consistently have elevated levels of antibodies targeted to periodontal bacteria

compared to sera of controls without CHD (Damgaard et al., 2017; Leishman et al., 2012). However, some studies have refuted this association (Boillot et al., 2016; de Boer et al., 2014). With such conflicting reports, a consensus on the association between serum antibody response targeted to periodontal bacteria and the risk of CHD is still lacking. We sought to critically explore the strength of this association through systematic review and meta-analysis approaches.

Review

Scope of review

The “Preferred Reporting Items for Systematic Reviews and Meta-Analyses” (PRISMA) guidance was followed. Using “Population Intervention/Exposure Comparator Outcome [P(I/E)CO]” criteria the review question was framed i.e. in the adult population (P), compared to the control participants (C), is elevated serum antibody response against periodontal bacteria (E) associated with an increased risk of coronary heart disease (O)?

Review strategy

Five databases, namely MEDLINE/PubMed, EMBASE, SCOPUS, WEB OF SCIENCE, Cochrane Controlled Trials Register were searched systematically. The syntax (***Table 1***) based on the National Library of Medicine Medical Subject Headings (MeSH) terms was constructed to search relevant articles up to April 2021. The reference lists of the selected articles were screened to identify additional relevant articles. As per the inclusion and exclusion criteria described below, two reviewers (CJ, RB) carried out literature searches. The inter-reviewer reliability was tested in an early pilot phase with a calibration exercise, which included 7 randomly selected (~10% of total articles). In case of disagreements, an arbitrator (WA) was available for mediation at each stage of the review. The titles were screened to shortlist relevant articles, whose abstracts were subsequently screened to select the manuscripts for full-text review. These steps were carried out by both reviewers, independently. No inter-reviewer disagreements were noted and the κ score was 0.99. The systematic review protocol is registered in the PROSPERO database (2017: CRD42017082259).

Inclusion and exclusion criteria

The following eligibility criteria were used: i) original research articles assessing serum antibody responses against periodontal bacteria in adults (age≥18 years); ii) studies reporting serum antibody response as exposure and coronary heart disease as an outcome; iii) cross-sectional, cohort and case-control studies; iv) studies published in the English language, and iv) studies conducted between January 1989 and April 2021. With regards to the selected period, the association between dental health and acute MI was first reported in 1989 (Mattila et al., 1989). Literature reviews, mini-reviews, dissertations, short commentaries, letters to the editor, *in vitro*

and animal studies were excluded. The PRISMA flow chart (***Fig. 2)*** illustrates the steps that were followed in this review.

Exposure and outcome

Serum sample positivity or elevated/high levels of antibody responses against periodontal bacteria were considered as the exposures of interest. In view of the variation in nomeclature used to identify coronary artery disease, we included studies with the following cardiac endpoints as outcomes of interest:coronary heart disease (CHD), coronary artery disease (CAD), ischemic heart disease (IHD), cardiovascular disease (CVD) that specifically leading to myocardial infarction (MI), acute coronary syndrome (ACS) and unstable angina pectoris..

Data Extraction and Quality Assessment

Two reviewers (CJ, RB) independently extracted the following information from each included study- first author and year of publication, country, study design, the total number of participants (N) with gender and age distribution, participants' characteristics, reported periodontitis status, antibody isotype and target, the outcome of interest, the seropositivity threshold or definition of high vs low antibody response, exposure-outcome relationship data, adjusted risk factors and study conclusions. For studies, where risk estimates were not reported, the number of seropositive and seronegative individuals in case-control groups was used to calculate these values. Both reviewers (CJ and RB) independently quality-assessed each included manuscript using the Newcastle-Ottawa Scale (***Table 2***). This is a star-based (☆) scale that assesses observational and non-randomised studies under three sub-sections- selection (maximum 5 stars), comparability (maximum 2 stars) and outcome (maximum 3 stars).

Statistical analyses

The interrater agreement at the screening and data extraction stages was assessed using κ statistic (Landis & Koch, 1977). For the meta-analysis, the extracted quantitative data [odds ratios (OR) and 95% confidence intervals] was analysed using RevMan software (RevMan 5.4.1, Cochrane Collaborative software, Baltimore, USA). The data were segregated according to antibody responses either against Pg or Aa. Publication bias was analysed using the Begg-Mazumdar rank correlation test of symmetry and presented in funnel plots. The pooled OR was calculated using the

inverse variance method in RevMan. Homogeneity across included studies was analysed using Q statistics, while heterogeneity was determined using the I^2 test (Higgins & Thompson, 2002). Where a high I^2 value (>50%) (Deeks, Higgins, & Altman, 2021) was detected, the sources of heterogeneity were investigated in a subgroup analysis by grouping included studies according to their common characteristics. In the sensitivity analysis, a cluster of studies sharing similar characteristics was dropped one at a time to evaluate the stability of the overall meta-analysis results.

RESULTS

Search results

A total of 752 published study titles were identified after systematically searching five databases. After removing the duplicates (n=7), 745 abstracts were screened based on the aforementioned inclusion-exclusion criteria. This yielded 20 publications that were eligible for the current systematic review. A review of the bibliographical list of these 20 articles provided no additional publications. The data extracted from the included 20 articles are shown in ***Table 3***. Nine out 20 articles were excluded from the meta-analysis for the following reasons- i) information on odds ratio or the number of seropositive vs seronegative individuals in case-control groups was missing (n =5) (Bohnstedt et al., 2010; Choi, Lee, Kim, & Choi, 2011; Leishman et al., 2012; Liljestrand et al., 2018; Paju et al., 2006); ii) the results were expressed as hazard ratio (n = 3) (Boillot et al., 2016; de Boer et al., 2014; Qi et al., 2020); iii) the risk estimate was reported as a relative risk (n =1) (Pussinen et al., 2005) (***Fig. 2***). Therefore, a quantitative synthesis of 11 publications was carried out in the present meta-analysis (Beck et al., 2005; Damgaard et al., 2017; Holmlund, Hedin, Pussinen, Lerner, & Lind, 2011; Hyvärinen et al., 2012; Lund Håheim, Olsen, Nafstad, Schwarze, & Rønningen, 2008; Łysek et al., 2018; Pussinen et al., 2003; Pussinen, Alfthan, Tuomilehto, Asikainen, & Jousilahti, 2004; Tabeta et al., 2011; Ueno et al., 2012; Yamazaki et al., 2007).

Qualitative synthesis

The Newcastle-Ottawa scale scoring revealed that 14 out of 20 studies scored ≥7, indicating the overall high quality for the majority of the included studies (***Table 2***). Since most of the included studies scored ≥7 scores, for the sensitivity analysis, we used 7 as a cut-off point to stratify included studies into two groups.

Population characteristics

The study designs of the included studies were as follows: cross-sectional (n=7) (Beck et al., 2005; Bohnstedt et al., 2010; Damgaard et al., 2017; de Boer et al., 2014; Leishman et al., 2012; Liljestrand et al., 2018; Pussinen et al., 2003), cohorts (n=9) (Boillot et al., 2016; Hyvärinen et al., 2012; Lund Håheim et al., 2008; Paju et al., 2006; Pussinen et al., 2004; Pussinen et al., 2005; Qi et al., 2020; Tabeta et al., 2011; Ueno et al., 2012) and case-control (n=4) studies (Choi et al., 2011; Holmlund et al., 2011; Łysek et al., 2018; Yamazaki et al., 2007). All studies were published between 2003 and 2020, which collectively included 15831 CHD patients and 8924 control participants. In this review, we categorised "nested case-control" studies as "cohort studies" since controls were part of the same cohort from which the cases were selected where exposure predates outcome (Ernster, 1994). The included studies were conducted in eleven different countries *i.e*. Finland (Hyvärinen et al., 2012; Liljestrand et al., 2018; Paju et al., 2006; Pussinen et al., 2003; Pussinen et al., 2004; Pussinen et al., 2005), Sweden (Holmlund et al., 2011)**,** Netherlands (de Boer et al., 2014), Japan (Tabeta et al., 2011; Ueno et al., 2012; Yamazaki et al., 2007), Australia (Bohnstedt et al., 2010; Leishman et al., 2012), USA (Beck et al., 2005; Qi et al., 2020), Norway (Lund Håheim et al., 2008), Denmark (Damgaard et al., 2017), Poland (Łysek et al., 2018), South Korea (Choi et al., 2011) and France (Boillot et al., 2016).

All study participants were adults with an age range of 40-80 years. Four out of 20 studies had only male participants (Lund Håheim et al., 2008; Pussinen et al., 2003; Pussinen et al., 2004; Pussinen et al., 2005). Fourteen out of 20 included studies used the independent dataset and the remaining 6 used data from the previously published large population-based studies (Beck et al., 2005; Liljestrand et al., 2018; Pussinen et al., 2003; Pussinen et al., 2005; Qi et al., 2020). Sixteen out of twenty included studies adjusted their results for the known risk factors of CHD (Beck et al., 2005; Bohnstedt et al., 2010; Boillot et al., 2016; Choi et al., 2011; Damgaard et al., 2017; de Boer et al., 2014; Holmlund et al., 2011; Hyvärinen et al., 2012; Leishman et al., 2012; Łysek et al., 2018; Pussinen et al., 2003; Pussinen et al., 2004; Pussinen et al., 2005; Yamazaki et al., 2007). None of the included studies provided a formal power calculation to support their sample size. All the selected articles defined the exposure and outcome measures clearly. CHD was defined based on

either a self-reported questionnaire (Damgaard et al., 2017; Lund Håheim et al., 2008) or physician-verified clinical records (Beck et al., 2005; Holmlund et al., 2011; Hyvärinen et al., 2012; Łysek et al., 2018; Pussinen et al., 2003; Pussinen et al., 2004; Tabeta et al., 2011; Ueno et al., 2012; Yamazaki et al., 2007) **(*Table 3*)**.

Techniques used to measure antibody responses against periodontal bacteria

All included studies used serum samples to measure periodontal antibody responses with only one exception (de Boer et al., 2014), which used plasma samples. All studies, except for one (Beck et al., 2005), utilised a standard Enzyme-Linked Immunosorbent Assay (ELISA) technique to quantify the serum antibody response against targeted periodontal bacteria. Beck *et al.* used a checkerboard immunoblotting technique (Beck et al., 2005). Eight studies (Damgaard et al., 2017; Holmlund et al., 2011; Hyvärinen et al., 2012; Liljestrand et al., 2018; Paju et al., 2006; Pussinen et al., 2003; Pussinen et al., 2004; Pussinen et al., 2005) quantified antibodies against the same set of periodontal bacterial antigens using the previously published multi-serotype ELISA protocol (Pussinen, Vilkuna-Rautiainen, Alfthan, Mattila, & Asikainen, 2002). In the remaining 5 studies, three used FDC381 and Su63 strains of Pg (Bohnstedt et al., 2010; Tabeta et al., 2011; Yamazaki et al., 2007), two used Pg ATCC 33277 and Aa ATCC 33384 strains (Lund Håheim et al., 2008; Ueno et al., 2012). Antibody targets in two other studies included: Pg-HSP60 peptide (Choi et al., 2011) and Pg-gingipain (Łysek et al., 2018). Unless stated otherwise, the strain of Pg or Aa used as a target in immuno-assays was not specified.

Quantitative synthesis

The majority (n=10) of the studies were focused on comparing anti-Pg responses in CHD patients and control subjects, followed by anti-Aa IgG antibody responses in 6 studies. A minority of shortlisted studies evaluated anti-Pg serum IgA levels (n=4) (Pussinen et al., 2004; Pussinen et al., 2005; Tabeta et al., 2011) and anti-Aa serum IgA levels (n=3) (Hyvärinen et al., 2012; Pussinen et al., 2004; Pussinen et al., 2005). Therefore, the scope of this meta-analysis was narrowed to assess the association of serum anti-Pg as well as anti-Aa IgG antibody responses and CHD risk. The studies were stratified into the following two groups, where the first group consisted of the studies reporting an association between anti-Pg serum IgG antibodies and CHD (n=10). Begg- Mazumdar rank test analyses showed the

absence of publication bias (p=0.39) as shown in funnel plots (***Fig. 3A***). These studies had a low heterogeneity [(Q= 11.7, p= 0.47) and I^2= 5.8%)]. However, variations were observed in the included studies with regards to the population characteristics and number of adjusted risk factors/confounders, which prompted us to use a random-effects model. The forest plot using a random model revealed a pooled OR of 1.23 (95% CI: 1.09-1.38, p=0.001) (***Fig. 3B***) that suggest a positive significant association between high serum anti-*Pg* IgG response and CHD. In pooled OR analyses, random vs fixed-effect modelling disclosed no significant differences (***Fig. 3B***). The second group comprised of the studies reporting an association between high anti-Aa serum IgG antibodies and CHD (n=6). Due to the presence of a moderate level of heterogeneity [(Q= 13.1, p= 0.04), I^2= 54.2%], a random-effects model was adopted to calculate a pooled odds ratio. Begg-Mazumdar rank test analyses of these 6 studies disclosed no evidence of publication bias (p=0.45) as shown in funnel plots (***Fig. 4A***). The forest plot generated by random modelling disclosed a pooled OR of 1.25 (95% CI: 1.04-1.47, p= 0.0004) (***Fig. 4B***). For the association between high anti-Aa serum IgG antibodies and CHD, the random vs fixed-effect modelling yielded no significant differences with the pooled OR estimates. In the sensitivity analysis, the pooled risk estimates in both meta-analyses did not differ significantly (p>0.05), even after removing the cluster of studies according to their common characteristics, such as- i) study design, ii) use of the multi-serotype ELISA, iii) OR adjusted for the risk factors and iv) the Newcastle-Ottawa study quality scores (≤7 vs >7) (**Table 4**). A subgroup analysis was performed to identify the sources of heterogeneity, which was detected in the studies assessing an association between anti-Aa antibody response and CHD. The studies were grouped using the same set of common characteristics as described earlier and the results revealed that each subgroup contributed to the overall heterogeneity (***Table 5***).

Discussion

According to the studies included in the present systematic review, CHD patients consistently showed higher levels of circulating antibodies against periodontal bacteria compared to controls. After combining the results of 11 studies, the meta-analyses disclosed a modest risk increase (1.2 times higher) of CHD for the subjects

with higher anti-*P. gingivalis* or anti-*A. actinomycetemcomitans* IgG antibody levels compared to individuals with antibody response defined as low or negative.

The scope of this review covered the association between antibody response against any bacterial species commonly associated with periodontitis and coronary heart disease. The literature search revealed that the majority of the studies examined antibody responses against two species, namely *P. gingivalis* and *A. actinomycetemcomitans* in CHD patients. Our systematic searches shortlisted 20 such studies for this systematic review. Of which, the majority (70%) of them scored ≥7 on the Newcastle-Ottawa scale. This scale is a star-based scoring system that assesses the quality of observational studies. The number of stars received by a study reflects its overall quality with the highest possible score is 10 (Stang, 2010). Further, 10 studies that reported an association between serum anti-Pg antibodies and risk of CHD and 6 studies that reported an association between serum anti-Aa antibodies and risk of CHD qualified for the meta-analyses. The heterogeneity between the studies assessing an association between serum anti-Pg antibodies and CHD (n=10) was very low as measured by I^2 (5.8%), which allowed us to derive a pooled odds ratio (Higgins & Thompson, 2002). It became evident that the cumulative effect size was influenced primarily by the large population-based studies. On this basis, the study by Lund Håheim L *et al.* was assigned the highest weight (27%), given its larger sample size (548 CHD patients and 625 control) (Lund Håheim et al., 2008). This was followed by the studies by Beck *et al.* (Beck et al., 2005) and Pussinen *et al.* (Pussinen et al., 2003). The remaining 7 studies were assigned 30% of the total weight. Yamazaki *et al.* had the smallest share of the overall weight (0.4%) because of its smallest sample size (51 CHD patients and 37 controls) (Yamazaki et al., 2007). In all except 4 studies, confidence intervals crossed the value of 1 (Beck et al., 2005; Pussinen et al., 2003; Tabeta et al., 2011; Yamazaki et al., 2007). Nonetheless, the pooled odds ratio of 1.23 was significant and had a relatively narrow confidence interval (1.09-1.38). However, the results of this meta-analysis should be interpreted with caution, given that the ORs of the two larger studies (Lund Håheim et al., 2008; Pussinen et al., 2003) were not adjusted for any known cardiovascular risk factors. In a sensitivity analysis, none of the subgroups altered findings of the pooled OR, indicating stability and robustness of the findings. The pooled OR with cohort studies, studies that utilised multiserotype

ELISA, studies that adjusted their results for more confounders (>6) and high-quality studies increased marginally in the sensitivity analysis. The significance of this marginal increase is at best doubtful, given the wide confidence intervals and it is likely due to the small number of studies in each subgroup. The Begg-Mazumdar rank correlation test did not disclose publication bias. This finding of the publication bias should be interpreted with caution, owing to the small number of studies included in the meta-analysis.

Amongst the 6 studies that assessed an association between serum anti-Aa IgG antibody levels and CHD, a random-effect model assigned a relatively similar share of the total weight to all the included studies. Only one study received the lowest share (3%) of the total weight due to its relatively smaller sample size (63 cases and 63 controls) (Pussinen et al., 2004). All studies, except for one (Beck et al., 2005), showed no significant results since the confidence interval crossed the line of no effect, likely due to the small sample sizes. Even though the effect sizes of all included studies showed a wide variation, we observed a pooled odds ratio of 1.25 with a relatively narrow confidence interval (1.04-1.47). The included studies had a moderate level of heterogeneity (I^2=54.2%), which was due to the type of study design, type of ELISA method and the number of adjusted cardiovascular risk factors, as disclosed by the sub-groups analysis. We speculate that the diversity could also have arisen from the differences in the study population, geographic location, the definitions of seropositivity, the cut-offs for high vs low antibody responses and inclusion of edentulous subjects. The effect of these covariates could not be assessed as the data relating to these factors were not reported. Similar to above, a sensitivity analysis revealed stability of the overall pooled OR in the fixed as well as the random-effects model. The cohort study design and the studies that used multi-serotype ELISA increased OR marginally but the associated CI interval widened too. The wider CI can be explained by the presence of a small number of studies in each aforementioned subgroup. The funnel plot and the Begg-Mazumdar rank correlation test confirmed the absence of publication bias. These results should be interpreted with caution since the number of selected studies is relatively small.

The included studies demonstrated methodological inconsistencies with regards to the diverse approaches employed in the immunoassays to measure antibody responses against periodontal bacteria. Lack of standardisation of the cut-off value

to define seropositive/seronegative or high/low IgG antibody levels was noted amongst included studies. In this context, the following differences were observed- i) five studies (Damgaard et al., 2017; Holmlund et al., 2011; Hyvärinen et al., 2012; Pussinen et al., 2003; Yamazaki et al., 2007) defined 'high antibody response' values exceeding the antibody level plus 1.5 times the standard deviation value of the periodontally healthy subjects, 2 studies compared the fourth versus first quartile values of the antibody titres (Lund Håheim et al., 2008; Pussinen et al., 2004), while 3 studies compared the third versus first tertile values of the antibody titres in the given cohort (Łysek et al., 2018; Tabeta et al., 2011; Ueno et al., 2012); all of which represent elevated antibody responses, ii) variation was observed in the definition of 'periodontally healthy' controls, which were used as a reference to calculate the cut-off value. No attempt has been made by us either to define or to standardise the cut-off value of high versus low seroreactivity, we relied on the respective authors' definitions. For the studies that did not report risk estimates for the association between anti-PG/anti-Aa and CHD risk separately, the categorisation of high versus low seroreactivity, as defined by the respective authors, was used to calculate the missing odds ratios (Lund Håheim et al., 2008; Pussinen et al., 2003).

Another critical problem is that both Pg and Aa display a wide clonal diversity (How, Song, & Chan, 2016; Nørskov-Lauritsen, Claesson, Birkeholm Jensen, Åberg, & Haubek, 2019; Ready et al., 2008). Therefore, it is important to consider the use of an antigen that is representative of the clonal types retrieved in the study population. In some studies, this was partly tackled by the multiserotype-ELISA protocol that was used in 5 out of 11 studies (Damgaard et al., 2017; Holmlund et al., 2011; Hyvärinen et al., 2012; Pussinen et al., 2003; Pussinen et al., 2004). This assay includes a mixture of 5 strains of Pg and 3 strains of Aa, developed by Pussinen et al. in 2002 (Pussinen et al., 2002). Amongst the included studies, other differences in ELISA experimental conditions were the type of antigen used to coat the plate, choice of fluorescence tagged-secondary antibodies, the wavelengths used to detect the optical signal. Few studies reported either combined IgG/IgA responses against periodontal bacteria (Liljestrand et al., 2018; Pussinen et al., 2003) or combined serum IgG against a group of different species of periodontal bacteria (Qi et al., 2020). In our view, antibody responses should be reported separately according to antibody isotype and targeted bacterial species. This approach will more likely

disclose the contribution of different antibody isotypes and periodontal bacterial diversity in coronary atherogenesis. Inconsistencies noted regarding antibody detection techniques limit our ability to comment on the precise cut-off for anti-Pg and anti-Aa serum IgG antibody levels, beyond which the risk of CHD is higher. The levels of serum anti-Pg and anti-Aa antibody responses may have been confounded by clinical variables such as periodontitis status, past periodontal therapy, number of teeth. A minority of the included studies reported data on periodontitis prevalence and periodontal parameters. However, amongst the included studies, a high level of heterogeneity was observed in relation to- i) periodontitis prevalence in the study groups, ii) periodontal clinical parameters. For example, Yamazaki et al. reported periodontal status in terms of mean PPD comparison between case-control groups, while Holmlund et al. reported the presence of a number of pockets >4 mm. Moreover, the segregated data on antibody responses in the subgroups of periodontitis vs periodontally-healthy individuals included in CHD and control groups were not available. Therefore, due to either data unavailability or high heterogeneity of datasets, the correlative analysis between IgG antibody response and periodontitis severity status could not be performed. Future studies should address this critical aspect. Further, most of the included studies did not report data regarding bacterial counts in subgingival plaques. With regards to the added value of subgingival plaque sampling for bacterial count estimation, we acknowledge the logistic challenges and financial considerations associated with full-mouth subgingival plaque sampling. However, the pooling of single subgingival plaque samples from all quadrants is commonly used as a validated surrogate of periodontal bacterial burden at a given time point. Future studies should include information on this important aspect. Finally, three studies evaluated the prognostic role of anti-periodontal bacterial antibodies in CHD events at 1 year-follow-ups and found no significant association between the two (Boillot et al., 2016; de Boer et al., 2014; Qi et al., 2020). It is noteworthy that the cardiac endpoints in these studies were self-reported. Given the number of studies reporting results as HR were very few, their pooled effect (either HR) estimates could not be investigated using a meta-analysis approach. With regards to the remaining 6 cohort studies, one study with a 10-year follow-up reported that individuals with higher anti-periodontal antibody responses were at significantly higher risk for experiencing CHD events (Pussinen et al., 2005). The remaining 5 cohort studies had nested case-control study design. Since data on

exposed vs unexposed populations in these studies was unavailable, the relative risk of suffering from CHD events at longitudinal assessments could not be calculated. CHD doesn't fulfil the rare disease assumption and therefore, OR and RR values cannot be approximated. Future cohort studies should consider this critical point while reporting their results.

In closing, most studies used the overarching descriptor CHD as an outcome of interest. This term includes unstable angina, ACS and MI, which represent different clinical manifestations of the underlying coronary atherosclerotic disease, determined by the extent and severity of atherosclerotic plaques. For example, stable atherosclerotic plaques are associated with angina, whereas rupture of unstable atherosclerotic plaques leads to MI. Therefore, in this review, we were not able to assess the independent association of anti-Pg and anti-Aa responses with angina, ACS or MI.

Recommendations for future studies

1) Future studies should report on the quantification of periodontal bacterial species in subgingival plaque and their correlation with the respective anti-periodontal bacterial antibodies in the case-control groups.
2) The studies must also report data relating to the confounding factors that influence the subgingival periodontal bacterial colonisation since these can reasonably be expected to affect the corresponding serum antibody responses. The minimum variables which must formally be accounted for and reported are- age, gender, smoking, diet, diabetes, socioeconomic status, recent antibiotic therapy, oral hygiene practices, number of teeth present the use of periodontitis case definition, past periodontal therapy and number of past CHD events. Ideally, cases and controls should be matched for the known risk factors of periodontitis and CHD.
3) Larger population-based cohort studies with longer follow-ups are needed to confirm the effect size for the association between anti-PG/anti-Aa antibodies and CHD. Because CHD is not a rare disease, future cohort studies should report the risk estimates as RR at the end of their follow-ups.
4) Also, more studies are needed to ascertain the prognostic role of anti-PG/anti-Aa antibodies with CHD events or death due to CHD.
5) ELISA assays need to be standardised for-

i. representative antigen(s) that cover the clonal diversity of different periodontal bacteria present within a given population e.g. approach such as the multi-serotype ELISA appears to be well-validated and covers a range of periodontal bacterial strains, commonly associated with periodontitis.

ii. the cut-off to differentiate the individuals with high versus low antibody titres should be determined. For example, a receiver operator characteristic (ROC) curve method can be employed to determine this threshold/cut-off limit against clinical indicators of periodontitis/subgingival bacterial levels. Clearly, this would require validation in multi-centre large-population based studies.

6) Special attention should be given to the use of cardiac nomenclature. Given the differences in the pathophysiologies of angina, ACS and MI, it is best to apply stringent inclusion criteria to include patients with specific cardiac pathology and investigate its association with anti-periodontal bacterial antibody responses, independently.

7) The interventional studies should evaluate if antibody titre reduction can be achieved after successful periodontal therapy and whether this correlates with a reduced CHD risk in a longitudinal assessment.

Conclusions

The majority of studies identified by this systematic review showed that circulating IgG antibodies against *P. gingivalis* and *A. actinomycetemcomitans* were higher in CHD patients compared to controls with no history of CHD. A meta-analysis of the included studies reporting odds ratios revealed that the risk of suffering from CHD is 1.2 times higher in individuals with elevated serum anti-*P. gingivalis* or anti-*A. actinomycetemcomitans* IgG antibody levels to individuals with antibody response defined as low or negative. The modest risk increase of CHD in patients identified as either seropositive or with high serum antibody responses against periodontal bacteria should be interpreted with caution, in the view of methodological inconsistencies noted amongst included studies. In particular, we wish to draw special attention to the inconsistent consideration given to key confounding factors known to influence the colonisation of periodontal bacteria in the subgingival environment as well as the observed differences in immunoassay designs and interpretation of their results.

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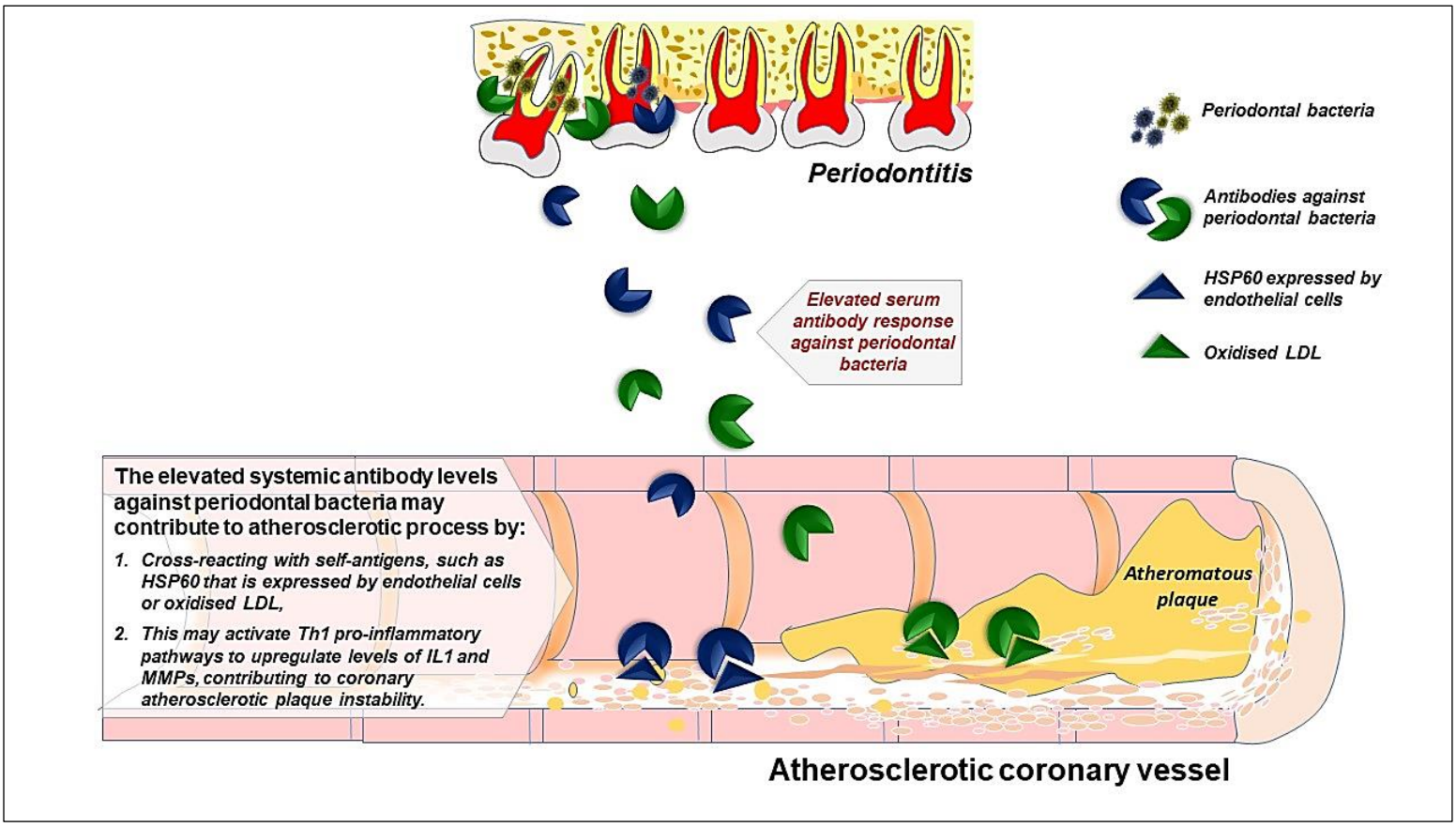
Figures

Figure 1: The cross-reactivity/molecular mimicry between serum antibody responses against periodontal bacteria and non-bacterial antigens that possibly contribute to the coronary atherosclerotic disease progression



The circulating antibodies against periodontal bacteria that are commonly implicated in the pathogenesis of periodontitis i.e. Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans cross-react with several non-bacterial self-antigens, such as endothelial heat shock protein-60 (HSP-60) and oxidised Low-density lipoprotein (LDL) cholesterol molecules. This interaction is believed to initiate and propagate the pro-inflammatory responses within atherosclerotic plaques.

Figure 2: Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram that was followed in the present review

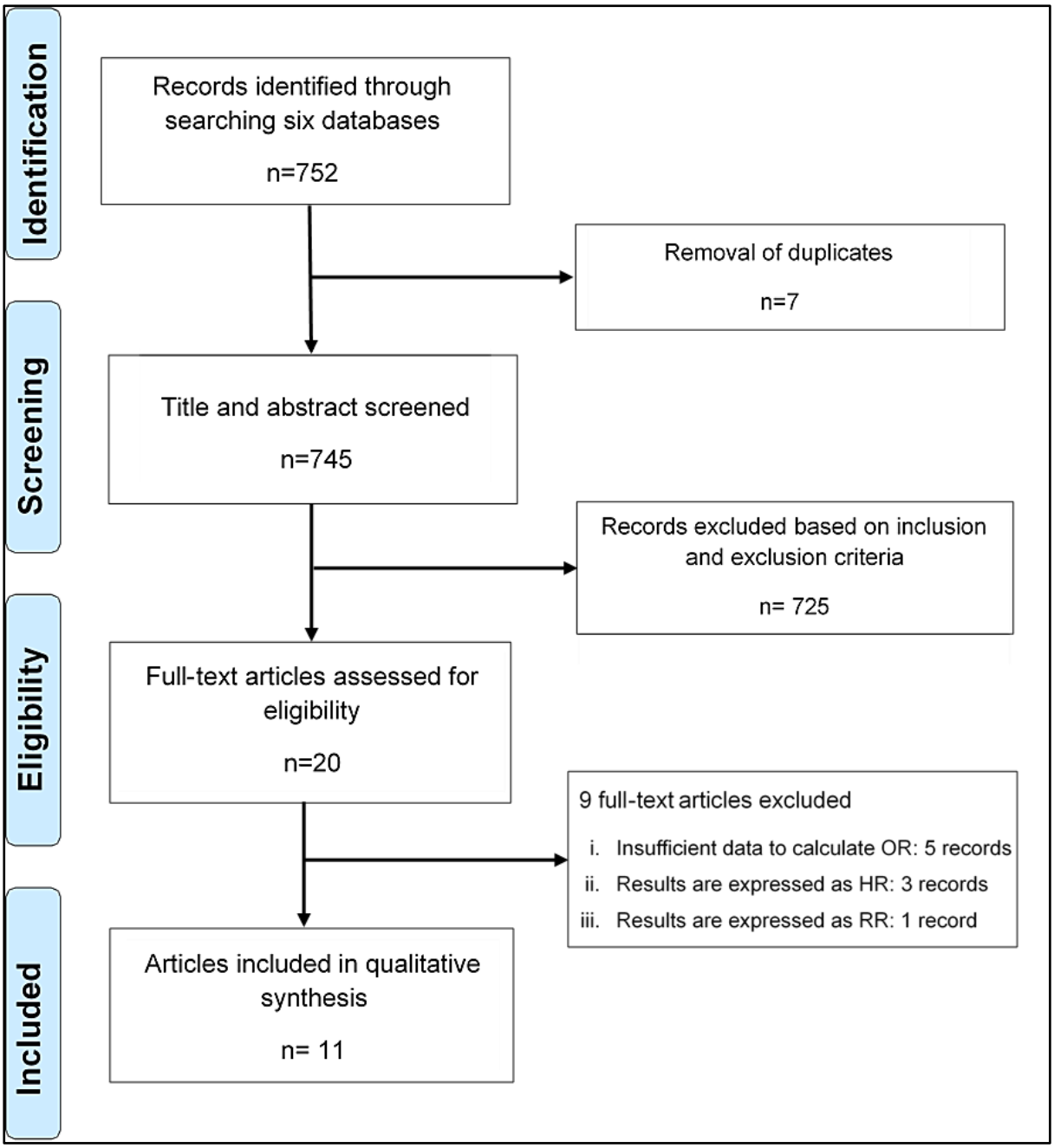
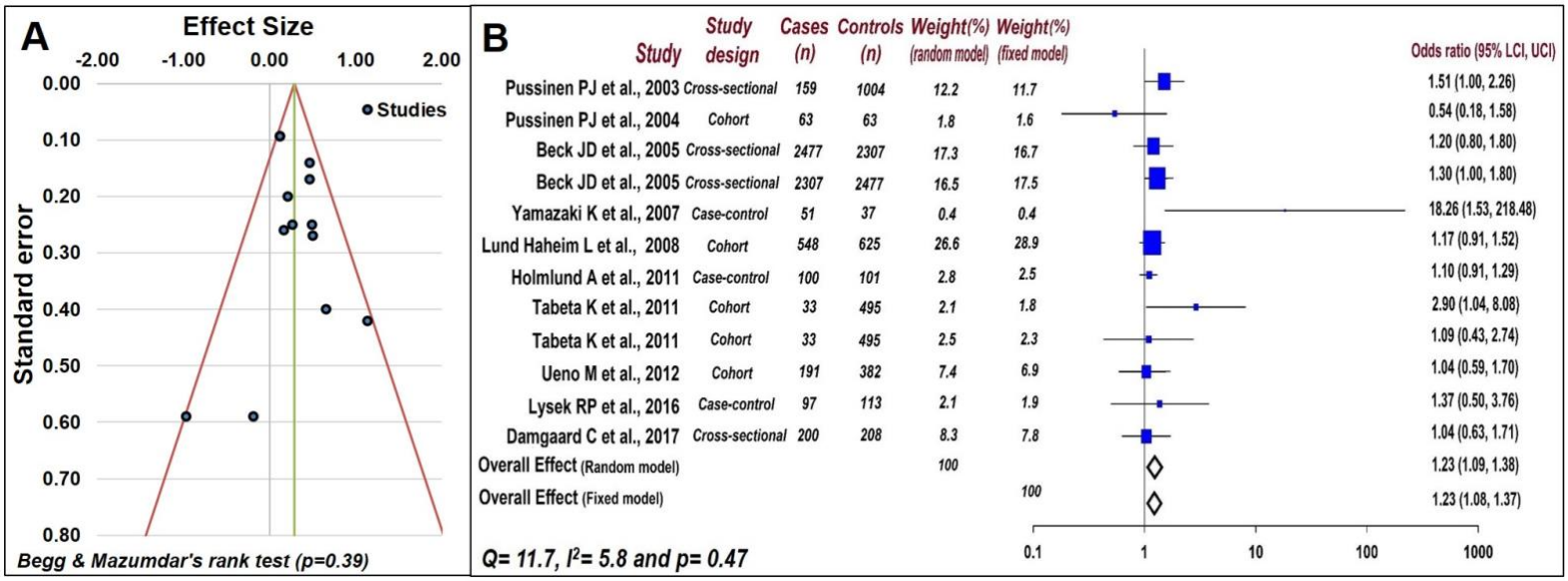
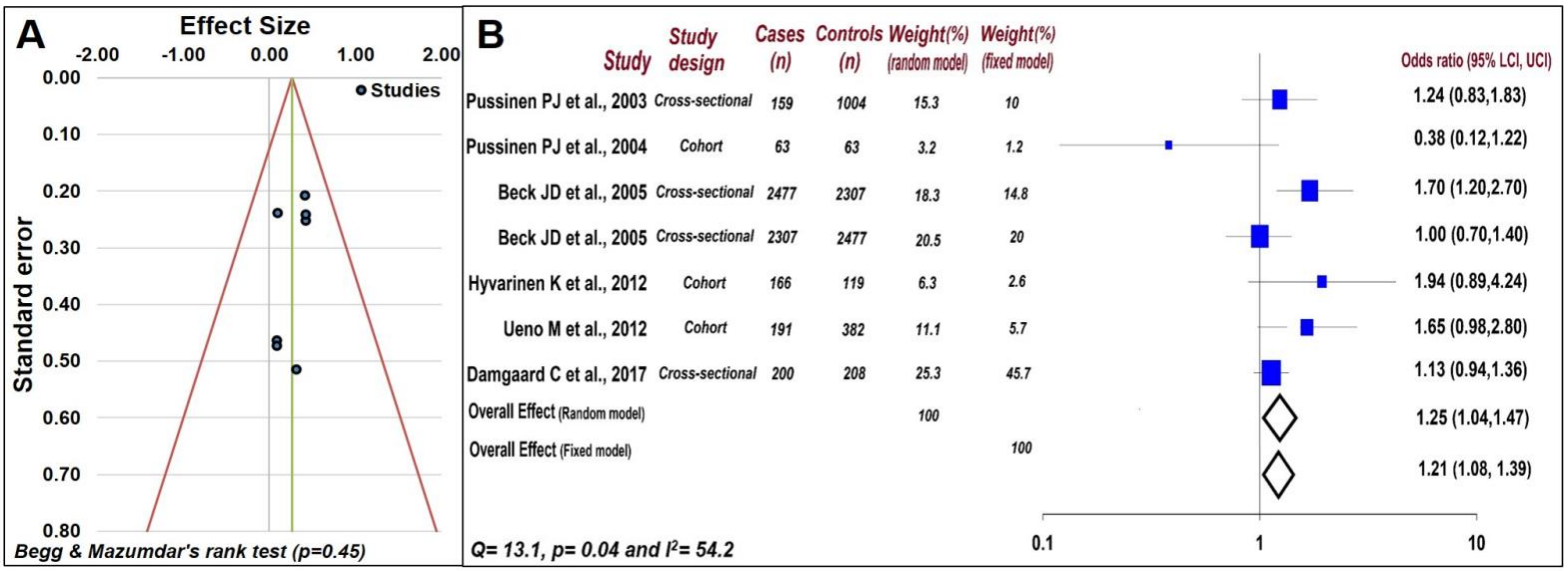


Figure 3: An association between serum IgG antibodies against *P. gingivalis* and risk of CHD (n=10)



A) Funnel plot illustrating the likelihood of publication bias among studies assessing an association between serum anti-Pg IgG antibody levels and CHD risk. B)Forest plot combining the results of 10 studies using random and fixed-effects models.

Figure 4: An association between serum IgG antibodies against *A. actinomycetemcomitans* and risk of CHD (n=6)



A) Funnel plot illustrates the absence of publication bias among studies assessing an association between serum anti-Aa antibody IgG levels and CHD risk. B) Forest plot combined the results of 6 included studies using the random and fixed-effects models.

Table 1: The MeSH terms used to search “Population intervention/exposure comparator outcome” question

PICO	**Search terms**
Population	Adult, young adults
Intervention/exposure	Microbiota, microbiome, the human microbiome, microbiology, bacteria, biofilm, dental biofilm, oral biofilm, dental deposits, dental plaque, *Porphyromonas gingivalis,* Bacteroides gingivalis, *Fusobacterium*, *Prevotella intermedia*, *Bacteroides intermedius, Aggregatibacter actinomycetemcomitans*, *Actinobacillus actinomycetemcomitans*, *Tannerella forsythia*, *Treponema denticola*, *Campylobacter rectus*, *Streptococcus sanguis*, Antibodies, Immunoglobulin G, Immunoglobulin A, Periodontal diseases, Periodontitis, Chronic periodontitis, Periodontal pocket, Alveolar Bone loss
Comparator	Healthy Volunteers, Non-Cardiac participants, Non-MI participants
Outcome	Cardiovascular diseases, Myocardial infarction, Coronary Artery Disease, Atherosclerosis, Coronary angiography, Coronary Thrombosis, Venous Thrombosis, Thrombosis, Thromboembolism
Search SYNTAX	[(microbiota OR microbiome OR "human microbiome" OR microbiology OR bacteria OR biofilm OR "dental biofilm" OR "oral biofilm" OR "dental deposits" OR "dental plaque" OR "Porphyromonas gingivalis" OR "Bacteroides gingivalis" OR fusobacterium OR "Prevotella intermedia" OR "Bacteroides intermedius" OR "Aggregatibacter actinomycetemcomitans" OR "Actinobacillus actinomycetemcomitans" OR "Tannerella forsythia" OR "Treponema denticola" OR "Antibodies" OR "Immunoglobulin G" OR "Immunoglobulin A") AND ("Periodontal diseases" OR Periodontitis OR "Chronic periodontitis" OR "Periodontal pocket" OR "Alveolar Bone loss") AND ("Cardiovascular diseases" OR "Myocardial infarction" OR "Coronary Artery disease" OR Atherosclerosis OR "Coronary angiography" OR "Coronary Thrombosis" OR "Venous Thrombosis" OR Thrombosis OR Thromboembolism) AND (“Case-Control Studies” OR “Cohort Studies” OR “Cross-Sectional Studies”)]

Table 2: Quality assessment of the included studies using the Newcastle-Ottawa Scale (n= 20)

Author, year	Selection	Comparability	Exposure/ Outcome	Score
Pussinen PJ et al., 2003	****	**	**	8
Pussinen PJ et al., 2004	****	**	*	7
Beck JD et al., 2005	****	**	*	7
Pussinen et al., 2005	****	**	*	7
Paju et al., 2006	****	**	*	7
Yamazaki K et al., 2007	***	**	*	6
Lund Håheim L et al., 2008	***	**	**	7
Bohnstedt et al., 2010	***	**	*	6
Tabeta K et al., 2011	****	**	**	8
Choi J et al., 2011	***	*	*	5
Holmlund A et al., 2011	***	**	**	7
Ueno M et al., 2012	***	**	*	6
Hyvärinen K et al., 2012	**	**	**	6
Leishman et al., 2012	***	-	**	5
de Boer et al., 2014	***	**	**	7
Boillot et al., 2016	****	**	**	8
Liljestrand JM et al., 2017	***	**	**	7
Damgaard C et al., 2017	***	**	**	7
Łysek RP et al., 2018	***	**	**	7
Qi J et al., 2020	***	**	**	7

Table 3: Characteristics of the studies included in this review (n=20)

Author and year	Country	Study design	Total number of participants (N), gender and age distribution	Participant characteristics	Reported periodontitis status	Antibody isotype and target	Outcome of interest	Seropositivity threshold or definition of high vs low antibody response	Exposure-outcome relationship data	Adjusted cardiovascular risk factors	Brief conclusions
Pussinen PJ et al., 2003	Finland	Cross-sectional	N=1163. All men. CHD cases (n=159), mean age 66.6±6.24 years and controls (n=1004), mean age 60.8±8.51 years	Patients with a history of previous CHD events were included.	Not reported.	Serum anti-Aa and anti-Pg IgG antibodies	CHD, based on the following symptoms: ECG ischemic changes, coronary angiography findings, or a history of previous CHD events as verified by an expert physician.	Mean absorbance value plus 1.5×SD of periodontally healthy subjects	Serum anti-Aa IgG antibodies, OR=1.237 (0.8301–1.834), *p*=0.02; Anti-Pg IgG antibodies, OR=1.506 (1.003-2.261), *p*=0.01	None	Seropositivity to Pg was associated with CHD.
Pussinen PJ et al., 2004	Finland	Cohort (Nested case-control)	N=126. All were men. MI cases (n=63), mean age 47.5±6.9 years; controls (n=63), mean age 47.8±7.0 years	None of the cases had experienced CHD in past.	Not reported.	Serum anti-Aa and anti-Pg IgA and IgG antibodies	Fatal MI based on Finnish National Death Certificate Register and non-fatal MI on the national hospital discharge records	The first, quartile values of antibody titres were used as reference	Serum anti-Aa IgG, OR=0.38 (0.12–1.22), *p*=0.08 and IgA OR=0.82 (0.26–2.58), *p*=0.2; Anti-Pg IgG, OR=0.54 (0.18–1.58), *p*=0.3 and IgA OR=3.30 (1.03–10.58), *p*=0.02	Smoking, serum cholesterol, blood pressure, BMI and diabetes	A significant association was found between serum anti-Pg IgA and future MI risk.
Beck JD et	USA	Cross-sectional	N= 4784. Individuals	None of the	Not reported.	Serum IgG antibody levels	Based on the hospital records	The median antibody	Serum anti-Pg IgG antibodies in	Age, sex, race/center,	Serum IgG levels against

al., 2005			ever-smoked were 2477 and individuals who never smoked were 2307. The age distribution is not reported.	participants had experienced CHD in past.		to 17 different oral microorganisms, including Pg and Aa	and health questionnaires	level for each bacterial species was used as a reference	individuals ever-smoked OR=1.3 (1.0–1.8), *p*>0.05 and those who never-smoked= 1.2 (0.8–1.8) *p*>0.05; Anti- Aa serum IgG antibodies in ever-smoked OR=1.0 (0.7–1.4), *p*>0.05 and for those who never-smoked OR=1.7 (1.2- 2.7), *p*<0.05	diabetes, hypertension, waist-to-hip ratio, HDL, LDL, and education	Aa were associated with CHD in non-smokers.
Pussinen et al., 2005	Finland	Cohort (10-year follow-up)	N= 910. All were men in the age range of 46 to 64 years, 109 subjects experienced a cardiac endpoint, MI, or CHD death.	At baseline, all participants were free from CHD.	Not reported.	Serum IgG and IgA class antibodies against Pg and Aa	Cardiac endpoint were i) deaths from the national death certificate register; ii) nonfatal AMI from the national hospital discharge data register.	Mean absorbance value plus 1.5×SD of the periodontally healthy subjects	Anti-Pg serum IgA RR= 1.5 (0.87–2.47), *p*= 0.01 and anti-Aa serum IgA RR=2.0 (1.21–3.33), *p*=0.02	Age, smoking, plasma fibrinogen, diabetes, medication for hypertension, socioeconomic status, and serum LDL+HDL cholesterol	High IgA antibody levels to both Pg and Aa were associated with CHD risk.
Paju et al., 2006	Finland	Cohort [average follow-up period of 519 days (range 138–924 days)]	N= 141. Clarithromycin group (n=70), 50 men and mean age 64.2±9.7 years. Placebo group (n=71), 46 men and mean age	The participants that had experienced CHD in past were included.	Overall prevalence of periodontitis was 54% (76 individuals).	Serum IgG and IgA antibody levels against Aa and Pg	The cardiac endpoints were CV death, myocardial infarction, unstable angina, or ischemic stroke. Two cardiologists verified patients files.	Mean absorbance value plus 1.5×SD of the periodontally healthy subjects	Segregated data on odds ratio or the number of seropositive individuals that specifically suffered MI is not reported.	Age, gender, current smoking, body mass index, diabetes, and hypercholesterolemia, hypertension	Aa and Pg seronegative patients met the specified cardiac endpoints less frequently in the clarithromycin versus the placebo group.

			63.4±10.6 years								
Yamazaki K et al., 2007	Japan	Case-control	N= 143. CHD cases (n=51), mean age 62.4±1.7 years and 46 men. Non-CHD individuals with periodontitis (n=55), mean age-47.2±1.7 years and 24 men. Controls (n=37) with no-CHD or periodontitis, mean age-48.6±1.5 years and 18 men.	Data on the history of previous CHD events in the case group is not reported.	Prevalence of periodontitis in each group not reported. Mean PPD (2.8±0.1 vs 2±0 mm) and CAL (3.04±0.02 vs 2.1±0.1 mm) in the CHD group was higher than controls (p<0.05).	Serum IgG antibody response against Pg Su63 and FDC381 strains	CHD cases were recruited from the Coronary Care Unit of Niigata City General Hospital.	Mean absorbance value plus 2×SD of the periodontally healthy subjects	Serum anti-Pg Su63 IgG antibody OR= 18.26 (1.53–218.48), p=0.02; Data for Anti-Pg FDC381 is not reported.	Age, gender	Antibody levels against Pg-Su63 were significantly associated with CHD.
Lund Håheim L et al., 2008	Norway	Cohort (Nested case-control) (28-year follow-up)	N=1173. All men. MI cases (n=548) and controls (N=625). The age range is 48–67 years.	The participants had not experienced CHD in past before 1973 (baseline).	Not reported.	Serum IgG antibody response against Pg ATCC 33277 and Aa ATCC 33384	Cardiac event information on the history of MI was self-reported, based on the responses obtained from a questionnaire.	For each species, the first quartile values of the antibody titres were used as reference	Serum Anti-Pg IgG levels OR=1.17 (0.91-1.52),p=0.01; Anti-Aa IgG levels OR= 0.66 (0.73-1.25). p=0.03	None	The level of antibodies against Pg and Aa is a stronger predictor of MI than non-specific inflammatory markers (e.g.C-reactive protein).

Bohnstedt et al., 2010	Australia	Cross-sectional	N= 701. the mean age of 50.14± 14.1 years and 367 were men	Data on the history of previous CHD events in the case group, prior to 3 years of enrolment, is not reported.	Overall prevalence of periodontitis is not reported. The proportion of individuals with ≤ 1 site ≥ 4 mm PPD was lower in the CVD group than the low CV risk group (44.4% vs 46.7%, respectively).	Serum IgG antibodies against the 6 strains of Pg (FDC 381, W50, SPBG, ATCC 33277, UQD605, and Su63)	293 Individuals that had experienced significant CV events (MI and angina) in the past 3 years were included. Details of CV events diagnosis/verification not reported.	Not reported	The odds ratio or the number of seropositive individuals for each strain is not reported.	None	Antibody levels against ATCC 33277, UQD605 and Su63 were higher CVD group than people with CVD.
Tabeta K et al., 2011	Japan	Cohort (Nested case-control) (5-year follow-up)	N= 594. IHD cases (n=33), mean age 69.7±7.7 and 58 men. Controls (n=495), the mean age of 69.7±7.6 years and 290 men.	The participants had not experienced IHD in past prior to 1998 (baseline).	Not reported.	Serum IgA and IgG antibodies against Pg (FDC381 and SU63 strains)	Medical charts were reviewed by the physicians for the diagnosis of IHD.	1st tertile of antibody level was used as a reference for each strain	Anti- Pg IgG SU63 OR=1.09 (0.43-2.74), *p*=0.8 and anti-Pg IgG FDC381 with OR= 2.9 (1.04-8.08), *p*=0.04	Age, gender, smoking, hypertension, diabetes mellitus, systolic blood pressure, serum total cholesterol and serum HDL-cholesterol	Antibody titres against both strains of Pg were not dose-dependently associated with the risk of IHD.
Choi J et al., 2011	South Korea	Case-control	N=60. Patient with periodontitis only (n=20),	Data on the history of previous	The prevalence of periodonti	Serum IgG titers against peptide 19 of Pg HSP60 and	Patients who underwent surgical intervention for	Not reported	The odds ratio or the number of seropositive	None	Serum anti-Pg HSP60 IgG titers were higher in

			patients with CAD and periodontitis (n=20), healthy controls (n=20). The age distribution is not reported.	CAD events are not reported.	tis in each group is not reported. Cases had higher mean PPD than controls (5.6±1.1 vs 2.8±0.04, respectively).	human HSP60 protein	atheromatous plaques.		individuals is not reported.		periodontitis and atherosclerosis+periodontitis patients than in controls. Peptide 19 of Pg HSP60 was an immunoreactive epitope leading to cross-reactivity.
Holmlund A et al., 2011	Sweden	Case-control	N=200. MI cases (n=100), mean age of 57.1±5.5 years and 79 men. Controls (n=101), mean 57.9±5.2 years and 81 men.	Data on the history of previous CHD/MI events in the case group is not reported.	The prevalence of periodontitis in each group is not reported. Cases had higher number of pockets >4 mm than controls [5 (1.0–13) vs 0 (0.0–3.0), resepctively].	Serum IgG and IgA class antibodies against Aa and Pg	Based on ECG changes in combination with serum creatinine kinase isoenzyme and troponin T	Mean absorbance value plus 1.5×SD of the periodontally healthy subjects	Serum anti-Pg IgG levels OR=1.10 (0.91-1.29), *p*=0.6; data for anti-Aa is not reported.	Age, gender, smoking, oral parameters-number of teeth <21; periodontal bone loss (no/minor, moderate and severe); >4 pockets >4 mm deep; and bleeding on probing on >20% the surfaces.	Serum anti-Pg IgG levels were associated with poor oral health and the risk of MI. No association was found between anti-Aa antibodies and MI.
Ueno M et al., 2012	Japan	Cohort (average follow-up	N=573 CHD cases (n=191),	The participants had not	Not reported.	Serum IgG antibodies against Aa	Medical charts were reviewed by the	1st tertile of antibody level was	Serum anti-Aa, antibodies-OR=1.65 (0.98-	BMI, smoking, alcohol intake, hypertension,	Serum anti-Pg and anti-Aa antibodies

		14-17 years)	mean age 56.7±7.7 years and 119 men. Controls (n=382), mean age 56.6±7.6 years and 238 men.	experienced CHD in past prior to 1990 (baseline).		ATCC 33384, Pg ATCC 33277, Pi ATCC 25611	physicians for the diagnosis of CHD.	used as a reference for each strain	2.8), *p*=0.06; Anti-Pg, OR=1.04 (0.59–1.7), *p*=0.9 and Anti-Pi, OR=1.89 (1.1-3.23), *p*=0.02	diabetes mellitus, exercise during leisure time and perceived mental stress.	were not related to CHD risk. In patients aged 56-69 years, the higher tertile level of anti-Pi antibodies was associated with a higher risk of CHD in a dose-response manner.
Hyvärinen K et al., 2012	Finland	Cohort (Nested case-control) (20 months follow-up)	N= 492. Mean age-63±9 and 492 were men. Cohort further divided into four groups- i) no significant CAD (n=119); ii) stable CAD (n=179); iii) ACS (n=166); iv) ACS-like, no CAD (n= 28)	Data on the number of participants that had experienced CAD in past prior to 2006 (baseline) is not reported.	Periodontitis prevalence in each group is not reported. Overall, serum anti-Aa IgA and IgG antibody levels were higher among patients with moderate-severe periodontitis	Serum IgG and IgA class antibodies against Aa	CAD was diagnosed based on ECG changes, chest pain with elevated levels of cardiac biomarkers, and >50% stenosis in at least one coronary artery.	Mean absorbance value plus 1.5×SD of the periodontally healthy subjects	Serum anti-Aa IgA antibodies, OR=3.13 (1.38-7.12), *p*=0.006 and anti-Aa IgG, OR=1.94 (0.89-4.24), *p*=0.09	Age, gender, BMI, hypertension, dyslipidemia, diabetes, and smoking	Serum IgA levels were significantly associated with an increased risk for CAD.

					than the periodontally healthy subjects (p<0.05 for both).						
Leishman et al., 2012	Australia	Cross-sectional	N=74. Mean age=61±8.27 and 61 men.	Data on the history of previous CVD events prior to 3 years of enrolment in the case group is not reported.	The overall periodontitis prevalence is not reported. The overall mean PPD was 1.74±0.38 mm.	Serum IgG antibodies against Pg, Tf, Fn, Aa and Pg hHSP60 and GroEL	The hospital admission for MI in the preceding 3 years as confirmed by reviewing medical records	Not reported	Anti-hHSP60 and anti-GroEL levels showed a significant correlation (r=0.39; p=0.001)	Not reported.	Elevated anti-hHSP60 levels were associated with poorer periodontal health in MI patients.
de Boer et al., 2014	Netherlands	Cohort (1-year follow-up)	N= 575. Mean age 61.6±11.3 years and 435 were men.	Data on the number of participants that had experienced past cardiac events prior to 2008 till 2011 (baseline) is not reported.	Not reported.	Plasma IgG and IgA against Aa, Pi, Pg and Tf	Cardiac endpoints were all-cause mortality, ACS or unplanned coronary revascularisation	1st tertile of antibody response for each bacterial species was used as a reference	High plasma concentrations of IgG and IgA against Pg HR=1.03 (0.55–1.92), p=0.94 and HR=0.87 (0.46–1.67), p=0.68, respectively. For anti-Aa IgG and IgA, HR= 0.91 (0.46–1.79), p=0.91 and HR= 1.19 (0.58–2.41), p=0.64., respectively. For anti-Tf IgG and IgA HR= 0.68	Age, gender, smoking and diabetes	Plasma levels of IgG and IgA against four major periodontal pathogens were not associated with coronary atherosclerotic disease.

									(0.36–1.27), *p*=0.23 and HR=1.13 (0.59–2.14), *p*=0.72. For ant-Pi IgG and IgA, HR= 0.63 (0.29–1.36), *p*=0.24 and HR= 1.18 (0.61–2.28), *p*=0.63		
Boillot et al., 2016	France	Cohort (1-year follow-up)	N= 975. Age and gender distribution are not reported.	390 of recruited 975 participants had experienced MI in past prior to 2005 (baseline)	Not reported.	Serum IgG and IgA against Pg, Aa, Pi and Tf	Fatal and non-fatal MI based on ECG changes in combination with serum creatinine kinase isoenzyme and troponin T.	Antibody levels are used as a continuous scale and HR is calculated 1 unit of antibody levels.	Serum anti-Pg IgG levels HR=0.96 (0.78 to 1.18), *p*=0.71; anti-Pg IgA levels HR=1.13 (0.90 to 1.42), *p*=0.31	Sex, age, diabetes, smoking, hypertension history of MI or heart failure, CRP levels	Serum anti-Pg and anti-Aa IgG antibodies were not associated with an increased risk of major adverse events in patients with a prior MI.
Damgaard C et al., 2017	Denmark	Cross-sectional	N= 576. CVD patients (n=200), mean age 62±10 years and 95 men. Periodontitis group (n=208), mean age 61±9 and 104 were men.	A number of CVD events that participants experienced is not reported.	The prevalence of periodontitis in each group is not reported. The periodontitis group had higher CAL values than the CVD	Serum level of IgG and IgG antibodies against Pg and Aa	The history of CVD events (MI, angina and atherosclerosis) was self-reported based on information obtained from a questionnaire.	Mean absorbance value plus 1.5×SD of periodontally healthy subjects	Serum anti-Pg IgG antibodies, OR=1.04 (0.63–1.71), *p*=0.6; anti-Aa IgG antibodies, OR=1.13 (0.94–1.36), *p*=0.18	Age, smoking, gender, alcohol consumption, overweight, and level of education	Serum anti-Pg and Anti-Aa IgG antibody levels were not associated with CVD when other risk factors were considered.

					group (3.48±1.09 vs 2.72±1.06 mm, respectively).						
Liljestrand JM et al., 2017	Finland	Cross-sectional	N = 505. No CAD (n=152), 46% men, age 61.2±9.2 years; Stable CAD (n = 184), 73.9% men, age 65.5±8.2 years and ACS (n=169), 72.2% men and age 62.9±9.6 years.	Data on the history of previous CAD events are not reported.	The prevalence of active periodontitis was higher in ACS patients compared to individuals without CAD (58.6% vs 46%, respectively). Anti-Aa and anti-Pg IgG and IgA levels were higher in the active periodontitis patients vs periodontally healthy individual	Serum levels of immunoglobulin A and G (IgA/IgG) against the whole-cell antigen of Aa, Pg, Pi, Tf, Cr and Fn	The coronary diagnosis was acquired from the coronary artery angiography, symptoms, an episode of typical chest pain, and elevated levels of cardiac biomarkers.	Serum IgA/IgG levels for the studied species were summed and expressed as total IgA/IgG burden. The first quartile values of the antibody titres were used as references.	Quartiles 2–4 of total IgA/IgG burden associated significantly with ACS (OR 1.98, 95%CI 1.13–3.47, *p*=0.017 for IgA; OR 1.84, 95%CI 1.04–3.23, *p*=0.035 for IgG).	Age, gender, smoking, dyslipidemia, hypertension, diabetes mellitus, BMI, and the number of teeth present	Elevated serum antibody levels to the studied periodontal bacterial species were associated with ACS.

					s (p<0.05 for all).						
Łysek RP et al., 2018	Poland	Case-control	N=220 individuals. MI cases (n=97), mean age 60.5 ± 8.7 years and 70 men. Controls (n=113), mean age-60.4 ± 8.7 years and 91 men.	Data on the history of previous MI events in the case group is not reported.	The prevalence of periodontitis in each group is not reported. Cases had a higher percentage of pockets >6 mm than controls (29.9% vs 17.7%, respectively).	Serum IgG antibodies against Pg gingipain	The definite clinical diagnosis of MI was based on the medical records	1st tertile of antibody response was used as a reference.	Anti-Pg antibodies, OR=1.37 (0.5-3.76), p<0.05	Sex, age, years of education, smoking, hypertension, hypercholesterolemia, body mass index, diabetes mellitus and number of teeth	Serum anti-Pg gingipain IgG antibodies were positively associated with MI risk.
Qi J et al., 2020	USA	Cohort (3-year follow-up)	N=6491 individuals. Males (n=2942); 78% of individuals were aged 40 to 64; CVD related-mortality in 810 individuals.	The participants had not experienced CVD in past.	Not reported.	Serum IgG against orange-red cluster (*Pm, Pi, Pn, Pg*). The red-green cluster (*Tf, Td, Aa, Ec, Sn, Vp, Cr*), yellow-orange cluster (*Si, So, Sm, Fn, Mm, Co*), and orange-blue cluster (*En, An*).	Underlying causes of death were recorded with validation from the death certificate.	For bacterial clusters, the first quartile values of the antibody titres were used as reference	The orange-blue cluster antibodies and CVD mortality (tertile 3 vs. tertile 1: HR=0.65, 95% CI=0.47 to 0.88, p= 0.0066).	Age, sex, race, educational level, income, smoking status, drinking status, body mass index (BMI), diabetes, hypertension, and annual dentist visits	Among all clusters, only orange-blue cluster antibodies, comprising E. nodatum and A. naeslundii were inversely associated with CVD mortality.

OR-odds ratio, CI- Confidence interval, CVD- Cardiovascular disease, CHD- Coronary heart disease, IHD- Ischemic heart disease, MI-Myocardial infarction, ACS- Acute coronary syndrome, PPD- Probing pocket depths, CAL- Clinical attachment levels, Pg- Porphyromonas gingivalis, Aa- Aggregatibacter actinomycetemcomitans, Pi- Prevotella intermedia, Tf- Tannerella forsythia, Fn- Fusobacterium nucleatum, Pm- Prevotella melaninogenica, Pn- Prevotella nigrescens, Ec- Eikenella corrodens, Sn- Selenomonas noxia, Vp- Veillonella parvula, Si-Staphylococcus intermedius, So-Streptococcus oralis, Sm-Streptococcus mutans, Mm- Micromonas micros, Capnocytophaga ochracea, En-Eubacterium nodatum, An-Actinomyces naeslundii, HR-Hazard ration, RR-Relative risk. The authors and the year of publication are marked by bold and italicized text to indicate the studies that were shortlisted for the meta-analysis.

Table 4: Sensitivity analysis evaluating the effect of the clusters of studies stratified based on the common characteristics on the pooled risk estimates in both meta-analyses

	Serum anti-Pg IgG antibodies and CHD association			***Serum anti-Aa IgG antibodies and CHD association***	
	Fixed model	***Random model***		***Fixed model***	***Random model***
	OR (95% CI), *p* value	OR (95% CI), *p* value		OR (95% CI), *p* value	OR (95% CI), *p* value
Overall (n=10)	1.23 (1.08 to 1.37), *p*=0.001	1.23 (1.09 to 1.38), *p*=0.001	***Overall (n=6)***	1.21 (1.08 to 1.39), *p*=0.04	1.25 (1.04 to 1.47), *p*=0.0004
Study design			***Study design***		
Cross-sectional studies (n=3)	1.18 (1.02 to 1.38), *p*=0.03	1.17 (1.02 to 1.41), *p*=0.03	Cross-sectional studies (n=3)	1.18 (1.05 to 1.31), *p*=0.02	1.21 (1.03 to 1.34), *p*=0.03
Cohort studies (n= 4)	1.29 (1.11 to 1.47), *p*=0.003	1.29 (1.11 to 1.47), *p*=0.001	Cohort studies (n=3)	1.43 (1.03 to 1.84), *p*=0.03	1.41 (1.06 to 1.81), *p*=0.04
Case-control studies (n=3)	1.22 (1.08 to 1.36), *p*=0.002	1.22 (1.1 to 1.34), *p*=0.001	Case-control studies (n=0)	None in this group	None in this group
ELISA technique			***ELISA technique***		
Multi-serotype ELISA technique (n=4)	1.24 (1.08 to 1.40), *p*=0.003	1.23 (1.1 to 1.41), *p*=0.001	Multi-serotype ELISA technique (n=4)	1.33 (1.14 to 1.52), *p*=0.03	1.35 (1.13 to 1.54), *p*=0.02
Other ELISA techniques (n=6)	1.20 (1.02 to 1.48), *p*=0.01	1.16 (1.01 to 1.50), *p*=0.01	Other ELISA techniques (n=2)	1.15 (1.04 to 1.31), *p*=0.04	1.13 (1.07 to 1.42), *p*=0.03
Number of adjusted CHD risk factors			***Number of adjusted CHD risk factors***		
Adjusted ≤6 CHD-related risk factors (n=3)	1.22 (1.04 to 1.40), *p*=0.001	1.17 (1.10 to 1.27), *p*=0.01	Adjusted ≤6 CHD-related risk factors (n=2)	1.22 (1.09 to 1.35), *p*=0.04	1.31 (1.07 to 1.55), *p*=0.02
Adjusted >6 CHD-related risk factors (n=7)	1.24 (1.03 to 1.45), *p*=0.02	1.20 (1.03 to 1.51), *p*=0.03	Adjusted >6 CHD-related risk factors (n=4)	1.09 (1.02 to 1.46), *p*=0.05	1.09 (1.03 to 1.49), *p*=0.05
Newcastle-Ottawa scale scores			***Newcastle-Ottawa scale scores***		
Scores ≤7 (n=2)	1.13 (1.03 to 1.43), *p*=0.04	1.19 (1.04 to 1.48), *p*=0.03	Scores ≤7 (n=2)	1.17 (1.04 to 1.30), *p*=0.03	1.17 (1.02 to 1.40), *p*=0.04

Scores >7 (n=8)	1.23 (1.09 to 1.38), *p*=0.002	1.20 (1.14 to 1.28), *p*=0.001	Scores >7 (n=4)	1.21 (1.11 to 1.22), *p*=0.002	1.21 (1.12 to 1.23), *p*=0.003

Table 5: Effect of each subgroup on the detected heterogeneity in the studies assessing an association between anti-Aa serum IgG antibodies and CHD risk (n=6)

		Statistics for each group		
Subgroup	**No. of studies**	**OR (95% CI)**	**P-value**	**I^2-value (%)**
Type of study design				
Cohort	3	1.18 (1.05-1.31)	0.005	50.1
Cross-sectional	3	1.54 (1.22-1.87)	0.0007	0
Use of the ELISA techniques				
Multi-serotype ELISA	4	1.23 (1.1-1.31)	0.002	45.1
Other techniques	2	1.43 (1.21-1.56)	0.03	32.2
No. of adjusted cardiovascular risk factors				
≤6	2	1.11 (1.10-1.27)	0.003	22.4
>6	4	1.36 (1.19-1.54)	0.0002	58.3