RESEARCH PROTOCOL

The association between coronary atherosclerosis and periodontitis: A clinical pilot study.

Version 2

27 March 2013

THE ASSOCIATION BEWTEEN CORONARY ATHEROSCLEROSIS AND PERIODONTITIS, A CLINICAL PILOT STUDY.

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Pharmacy	Not applicable for this study

PROTOCOL SIGNATURE SHEET

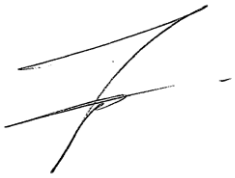
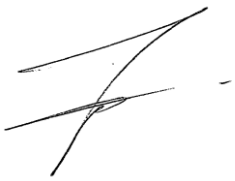
Name	Signature	Date
Sponsor or legal representative: Prof. dr. J. de Lange Head of Department of Oral and Maxillofacial Surgery, AMC		15/05/2013
Coordinating Investigator/Project leader Prof. dr. J. de Lange Head of Department of Oral and Maxillofacial Surgery, AMC		15/05/2013

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AMC	Academic Medical Center
ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
AR	Adverse Reaction
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority
CAC	Coronary Artery Calcium
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CCS	Coronary Calcium Score
CCT	Cardiac Computed Tomografy
CHD	Coronary Heart Disease
DSMB	Data Safety Monitoring Board
ECG	Electrocadiografy
eGFR	estimated Glomerular Filtration Rate
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
FMD	Flow Mediated Dilatation
GCP	Good Clinical Practice
HbA1c	Glycated hemoglobin
HDL	High Density Lipoprotein (cholesterol)
HR	Heart Rate
(hs)CRP	(high sensitive) C reactive Protein
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
IMT	Intima Media Thickness
LDL	Low Density Lipoprotein (cholesterol)
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
PISA	Periodontal Inflamed Surface Area
PPZ	Practice for Periodontology Zwolle (Dutch: Praktijk voor Parodontologie Zwolle)
(S)AE	(Serious) Adverse Event
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen
WHR	Waist to Hip Ratio

SUMMARY

Rationale:
Periodontitis is a chronic multi-causal inflammatory disease of the supportive tissues of the teeth with progressive loss of attachment and alveolar bone. Atherosclerosis is perceived today as a chronic inflammatory condition, and infectious diseases are believed to contribute to its pathophysiology. In recent years remarkable pathological and epidemiological relationships between periodontitis and atherosclerosis have been presented. Many previous studies use surrogate biomarkers in order to demonstrate the association between periodontitis and atherosclerosis. Unfortunately, more definitive cardiovascular parameters and endpoints are still lacking.

Objective:
The aim of this study is to determine if there is an association between the PISA score and the coronary calcium score. In other words, whether the presence and the extent of periodontitis is related to the presence and extent of coronary calcification as seen on the coronary calcium score..

Study design:
Cross-sectional pilot study

Study population:
50 human volunteers without known systemically diseases, 45-70 years old, who visited the Practice for Periodontology Zwolle (PPZ) for either recently diagnosed periodontitis or dental implants.

Main study parameters/endpoints:
All patients have to fill in questionnaires to gather data on their medical history, perceived health, parental history, lifestyle, socio-economic status and oral hygiene. A physical examination (Blood Pressure (BP), Heart Rate (HR), Body Mass Index (BMI), Waist to Hip ratio (WHR), Electrocardiogram (ECG)), a periodontal index, a standard dental panoramic radiograph, oral microbiological samples and blood test (hsCRP, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, eGFR, HbA1c, genotyping) will be obtained. Inflammatory burden of periodontal disease will be quantified with the Periodontal Inflamed Surface Area (PISA) score. To acquire data about the cardiovascular condition, we will obtain a Coronary Calcium Score by a CT-scan. The endothelial function will be measured employing “EndoScore” using the ENDOPAT.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:
The radiation exposure of a standard dental panoramic radiograph is estimated to be 0.010 mSv, comparable to 1 day background exposure to the natural radiation in The Netherlands. The radiation exposure of a cardiac CT-scan for coronary calcium scoring is estimated to be 1.5 mSv for each participant. This is comparable to 6 months background exposure to the natural radiation in The Netherlands.

1. INTRODUCTION AND RATIONALE

Cardiovascular disease is the leading cause of death and morbidity in the Western world. The underlying pathology, atherosclerosis, is a progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries. It constitutes the most important contributor to the growing burden of cardiovascular disease.[1] Over the past two decades, inflammation has emerged as an integrative factor for atherosclerosis. Inflammation can operate in all stages of this disease from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis.[2]

Periodontitis is a chronic multi-causal inflammatory disease of the supportive tissues of the teeth with progressive loss of attachment and alveolar bone. It is the most common oral disease, affecting 40-50% of the adults and approximately 10% of the population in its most severe form.[3] The first study that found positive epidemiological evidence for the association between periodontitis and atherosclerosis was in 1989 by Mattila *et al*.[4] Thereafter, in more recent years, remarkable pathological and epidemiological associations between these two diseases have been presented.[5] A very important topic, with regard to the high incidence of both diseases, their economic costs to society and the potential impact on public health, is risk modification and identification of therapeutic opportunities.[6]

Biological mechanisms for the association between periodontitis and coronary heart disease

Several pathophysiological pathways have been suggested to explain the association between periodontitis and atherosclerosis. These pathways involve both direct and indirect mechanisms.

Indirect mechanism: Increased level of systemic inflammation: Periodontitis is associated with increased levels of C-reactive protein (CRP), fibrinogen, tumor necrosis factor-α, IL-1, IL-6, IL-8 and other acute phase reactants.[7] These inflammatory reactants promote systemic inflammation and are associated with atherosclerosis. In case of systemic inflammation, endothelial cells stimulated by these inflammatory reactants increase their expression of various leukocyte adhesion molecules. Once adherent to the activated endothelial layer, the monocyte moves between the endothelial cells to penetrate into the innermost layer of the arterial wall and initiates an atherosclerotic lesion. Once resident in the arterial intima, monocytes acquire the morphological characteristics of macrophages, undergoing a series of changes that lead ultimately to foam cell formation. These foam cells are lipid-laden macrophages and characterize the early atherosclerotic lesion. Macrophages within atherosclerotic plaques also secrete a number of growth factors and cytokines involved in lesion progression and complication.[2]

Indirect mechanism: Increased platelet activation: Periodontitis is associated with increased p-selectin and platelet activation.[8] P-selectin functions as a cell adhesion molecule on the surfaces of arterial endothelial cells and activates platelets, in response to inflammation. Periodontopathogens are able to directly cause activation of endothelial cells and platelets.[9] Since platelet activation contributes to a pro-coagulant state and constitutes a risk for atherothrombosis, platelet activation in periodontitis may partly explain the epidemiological association between periodontitis and atherosclerosis.[8]

Direct mechanism: Invasion of periodontal pathogens into atherosclerotic plaques: Bacteremia that originates from the mouth is a common event that occurs multiple times a day while chewing and tooth brushing, especially in patients suffering gingivitis and periodontitis.[10] Periodontal pathogens (i.e. Porphyromonas gingivalis, Aggregatibacter *Actinomycetemcomitans*, Prevotella intermedia, Treponema denticola and Eikenella corrodens) enter the circulation via the gingival sulcus. These periodontal pathogens adhere to and invade in vascular endothelial cells. Infection of these endothelial cells by the periodontal pathogens (in particular Porphyromonas gingivalis) induces a procoagulant response that might contribute to formation of an atherosclerotic plaque.[10] Moreover, periodontal pathogens have been found in atherosclerotic plaques.[11]

Potential genetic mechanism: The recent identification of a shared genetic locus, ANRIL, for periodontitis and atherosclerosis/coronary heart disease, is a factor of unknown influence, but could be even more important than the above proposed biological mechanisms.[12] The function of ANRIL and its role in periodontitis and atherosclerosis is still lacking.

Epidemiological evidence for the association between periodontitis and atherosclerosis

Several epidemiological studies have confirmed the association between periodontitis and atherosclerosis. The first study that found positive epidemiological evidence for this association was in 1989 by Mattila *et al*.[4] Bahekar *et al*. recently summarized the subsequent studies in a systematic review revealing five prospective cohort studies (follow-up >6 years), five case-control studies and five cross-sectional studies. Meta-analysis of the five prospective cohort studies (86092 patients) indicated that individuals with periodontitis had a 1.14 times higher risk of developing coronary heart disease (CHD) than the controls (relative risk 1.14, 95% CI 1.074–1.213, $P < 0.001$). The case–control studies (1423 patients) showed an even greater risk of developing CHD (OR 2.22, 95% CI 1.59–3.117, $P < 0.001$). The prevalence of CHD in the cross-sectional studies (17724 patients) was significantly greater among individuals with periodontitis than in those without periodontitis (OR 1.59, 95% CI 1.329–1.907, $P < 0.001$).[13] The individual studies were well be adjusted for confounding factors, because of the extensive documented impact of many prevalent risk factors, shared by periodontitis and CHD. These shared risk factors include increasing age, male sex, race/ethnicity, education and socio-economic status, stress, smoking, alcohol abuse, diabetes mellitus and overweight.[14, 15]

Clinical evidence in the literature for the association between periodontitis and atherosclerosis

Observational studies using surrogate endpoints: Periodontitis can correctly be diagnosed and controlled by intra-oral examination (gingival bleeding, pocket-depth, loss of attachment and microbiological sampling or analysis) and dental X-ray (loss of alveolar bone). However, to strictly diagnose atherosclerosis, there is a need of invasive techniques such as angiography of the coronary arteries. Today, several surrogate biomarkers and imaging tools for atherosclerosis are in clinical and experimental use. Since inflammation has emerged as an integrative factor for atherosclerosis, epidemiological studies have found increased vascular risk in association with increased levels of inflammatory biomarkers such as cytokines (IL-6, TNF-α), cell adhesion molecules (P selectin) and acute-phase reactants (CRP, fibrinogen), which are elevated in periodontitis patients.[7] Growing evidence indicates

that elevated circulating inflammatory markers, in particular CRP, are predictors for an unfavorable course, independent of the severity of the CHD or inflammatory burden.[2] Paraskevas *et al*. showed in a meta-analysis of 10 cross-sectional studies that the weighted mean difference of CRP between periodontitis patients and controls was 1.56 mg/l (p < 0.00001).[16] Elevated levels of CRP (>2.1 mg/l) are associated with a higher incidence of acute thrombotic events including stroke and myocardial infarction. CRP levels >2.1 mg/l in healthy individuals may be associated with a chronic pro-coagulant state and they may serve as markers for an increased long-term risk of CHD.[17]

Besides, there are a number of other non-invasive surrogate subclinical markers of cardiovascular disease, focused on the endothelial function and arterial stiffness, including measurement of the carotid arteries, echocardiography, ankle-brachial index, flow-mediated dilation (FMD) in the brachial artery and pulse waveform analysis.[18] Söder *et al*. found significantly higher mean values of the common carotid artery intima-media thickness (IMT) and calculated intima-media area (cIMA) in patients with periodontitis than in controls, both at the right (P < 0.01 and P < 0.001, respectively) and left side (P < 0.001 for both variables).[19] Carotid IMT increase is associated with a raised risk of CHD.[20] Endothelial dysfunction precedes clinical manifestation of atherosclerosis. Flow-mediated dilation (FMD) of the brachial artery assesses the endothelial function and is decreased in subjects with atherosclerosis. Amar *et al*. displayed that subjects with advanced periodontitis had lower FMD compared with control patients (7.8 +/- 4.6% versus 11.7 +/- 5.3%, P = 0.005).[21]

Clinical trials concentrated on atherosclerotic risk reduction after periodontal treatment: Whether or not periodontal treatment reduces the risk for atherosclerosis or complications of atherosclerosis have not yet been established. The majority of the intervention trails, aimed to study this purpose, has examined the effect of periodontal treatment on markers of systemic inflammation or surrogate biomarkers of atherosclerosis. A recent meta-analysis on C-reactive protein in relation to periodontitis has indicated that periodontal treatment resulted in a weighted mean reduction in serum CRP of 0.5 mg/l (95% CI 0.08–0.93, p = 0.02). This reduction leads to clinical relevant improvements in systemic inflammation.[16] Tonetti *et al*. sought to assess the effect on intensive periodontal treatment on endothelial function measured by FMD of the brachial artery. FMD was greater, and thus improved, in the intensive-treatment group than in the control-treatment group 60 days after therapy (absolute difference 0.9%; 95% CI, 0.1 to 1.7; P = 0.02) and 180 days after therapy (difference, 2.0%; 95% CI, 1.2 to 2.8; P < 0.001). The degree of improvement was associated with improvement in measures of periodontal disease.[22]

Conclusion

The association between periodontitis and atherosclerosis is of great public health importance because of the high prevalence of both diseases and the potential impact on public health if risk modification or therapeutic opportunities could be identified. Previous studies use surrogate biomarkers in order to investigate the association between periodontitis and atherosclerosis. Unfortunately, more definitive cardiovascular parameters and endpoints are still lacking.

Several biological pathways have been suggested to explain this association; however, causal mechanisms are still not demonstrated. Polymorphisms in the ANRIL gene, which has been associated with both atherosclerosis and periodontitis, might be an important factor.

Several studies have evaluated the effects of periodontal treatment on endothelial function, in particular improvement of the cardiovascular condition through an increase of flow-mediated dilation (FMD) after periodontitis intervention has been observed. However, these

studies utilize the brachial artery, which is a surrogate for the condition of coronary arteries. Besides, only subjects with severe periodontitis have been included in these clinical trials of periodontal therapy and endothelial function improvement. Therefore, there is still no available evidence that periodontal treatment improves endothelial function in subjects affected by the more prevalent forms, i.e. slight or moderate periodontitis. Studies exploring the effects of periodontal therapy in atherosclerotic patients with periodontitis are needed because treatment studies with periodontitis patients without atherosclerosis have shown benefits for cardiovascular system. We designed studies focused on more definitive quantification of the link between periodontitis and atherosclerosis, and the benefits of periodontal intervention in atherosclerotic patients. Further, the prevalence of ANRIL risk alleles will be linked with inter-individual variation in response to periodontal therapy. Finally in all participants microbiological samples will be taken for the determination of the oral microbiome using pyrosequencing, to determine a possible specific “fingerprint” of oral microbiome associated with atherosclerosis.

Relevance for science, technology or society

This study may provide clinical evidence that periodontitis is a risk indicator for atherosclerosis. In the future, patients suffering from periodontitis might be screened for atherosclerosis. Early diagnosis of atherosclerosis, coupled with secondary prevention such as life-style changes and targeted medication, has been shown to have a favourable effect on the course of the disease. Besides, diagnosed atherosclerotic patients if suffering from periodontitis are likely to benefit from periodontal treatment.

2. OBJECTIVES

The aim of this study is to determine if there is an association between the PISA score and the coronary calcium score. In other words, whether the presence and the extent of periodontitis is related to the presence and extent of coronary calcification as seen on the coronary calcium score..

3. STUDY DESIGN

This is a cross-sectional (pilot) study, coordinated by the department of Oral and Maxillofacial Surgery of the Academic Medical Center (AMC), and the Department of Periodontology, ACTA, Amsterdam. Patients from the Practice for Periodontology Zwolle will be recruited. The department of Cardiology from the Isala Clinics will obtain the physical examinations, blood test, arterial stiffness test and the Coronary Calcium Score (CCS).

4. STUDY POPULATION

4.1 Population (base)

Patients who visited the Practice for Periodontology Zwolle for either recently diagnosed periodontitis or dental implants.

4.2 Inclusion criteria

- Age 45-70 years
- Male and Female
- All ethnicities
- ≥ 10 teeth

4.3 Exclusion criteria

- Edentulous
- Known cardiovascular diseases
- Known Diabetes Mellitus
- Known autoimmune disorders
- Known immunosuppressive disorders

4.4 Sample size calculation

Not applicable. There is no data available on which a sample size calculation can be based. This is a pilot and feasibility study with 50 participants to determine the study population size needed for a full study.

5. TREATMENT OF SUBJECTS

Not applicable for this study.

6. INVESTIGATIONAL MEDICINAL PRODUCT

Not applicable for this study.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter/endpoint

Periodontal Inflamed Surface Area (PISA): [23]
Periodontal inflamed surface area (PISA) quantifies the amount of inflamed periodontal tissue in square centimetres and is supposed to quantify the inflammatory and infectious burden resulting from periodontitis. PISA will be calculated after extensive periodontal examination including periodontal probing pocket depth (PD), gingival recession, plaque score, and bleeding on probing (BOP) measurements by trained and calibrated examiners. All measurements will be performed on all teeth, on six sites per tooth using a manual periodontal standard probe. Clinical attachment loss (CAL) will be defined as the distance from the cementoenamel junction to the bottom of the pocket/sulcus and calculated as the mathematical sum of the PD and gingival recession measurements. Measurements will be made in millimeters and will be rounded off to the nearest millimeter. BOP will be recorded as either present or absent within 30 seconds of probing at six sites per tooth. Plaque score will be defined as being present or absent at six points on each tooth. The number of missing teeth will also be recorded.

Coronary Calcium Score (CCS): [24]
Quantifying the amount of coronary artery calcium (CAC) with cardiac CT has been widely accepted as a reliable non-invasive technique for predicting risk of future cardiovascular events. Although CT-detected coronary calcification only allows evaluation of the calcified component of coronary plaques, a good correlation has been found between the Coronary Calcium Score and the total amount of coronary atherosclerosis as assessed by intravascular ultrasound and histology. Thus, the CCS is a good measure for the total amount of coronary atherosclerosis, including stable and vulnerable plaques. Interestingly, the CCS has been found to have a closer association with total coronary plaque burden compared with the presence of luminal stenosis. An increasing amount of coronary calcification is correlated with the probability of obstructive coronary artery disease by invasive coronary angiography.

Endothelial dysfunction (EndoScore): [25]
Endothelial dysfunction has been recognized as the critical junction between risk factors and clinical disease. It is the earliest detectable stage of cardiovascular disease. EndoPAT is a medical device for noninvasive endothelial function assessment. EndoPAT is based on noninvasive Peripheral Arterial Tone (PAT) signal technology. It measures endothelium-mediated changes in vascular tone using unique bio-sensors placed on the fingertips. These changes in arterial tone are elicited by creating a down-stream hyperemic response induced by a standard 5-minute occlusion of the feeding artery (using a standard blood pressure cuff). When the cuff is released, the surge of blood flow causes an endothelium-dependent Flow Mediated Dilatation (FMD). The dilatation, manifested as Reactive Hyperemia, is captured by EndoPAT as an increase in the PAT Signal amplitude. A post-occlusion to pre-occlusion ratio is automatically calculated by the EndoPAT software, thus providing the EndoScore. Measurements from the opposite arm are used to control for concurrent non-endothelial dependent changes in vascular tone. EndoPAT tests can be carried out in both the office and hospital settings, with patients positioned either sitting or supine. The test takes 15 minutes to complete, is very easy to perform, and is both operator and interpreter independent.

7.1.2 Secondary study parameters/endpoints

Reynolds risk score: [26]
The Reynolds Risk Score is designed to predict the risk, in non-diabetic patients, of having a future heart attack, stroke, or other major cardiovascular event in the next 10 years. This model includes age, current smoking, parental history of a cardiovascular event, <age 60 years, blood pressure, hs-CRP and total and HDL cholesterol.

Framingham risk score: [27]
The Framingham risk score is one of the most commonly used risk-estimation systems, which enables clinicians to estimate cardiovascular risk in asymptomatic patients. It is calculated using traditional risk predictors, including age, gender, total cholesterol, high-density lipoprotein cholesterol, smoking status, and systolic blood pressure, and is represented as a 10-year risk score for the prediction of coronary artery disease events.

7.1.3 Other study parameters

Questionnaires:

- Age
- Sex
- Ethnicity
- Socio-economic status
- Medical history and medication (history)
- Parental history
- Life-style (smoking, alcohol, drugs, stress)
- Oral hygiene

Physical examination:

- Blood Pressure (BP)
- Heart Rate (HR)
- Body Mass Index (BMI)
- Waist to Hip Ratio (WHR)
- Electrocardiogram (ECG)

Biochemical parameters:

- High sensitive C-reactive Protein (hsCRP)
- Total cholesterol
- High Density Lipoprotein (HDL) cholesterol
- Low Density Lipoprotein (LDL) cholesterol
- Triglycerides
- Estimated Glomerular Filtration Rate (eGFR)
- Glycated Hemoglobine (HbA1c)

Extra parameters for coupled studies:
One blood sample for DNA analysis of risk genes for both periodontitis and coronary heart disease.
Oral rinse sample for microbiological analysis of the oral microbiome peformed by pyrosequencing of 16Smicrobial DNA.

7.2 Randomisation, blinding and treatment allocation

Not applicable for this study.

7.3 Study procedures

Baseline
Location: PPZ
Procedures:

- **Patient selection**
- Providing **information**

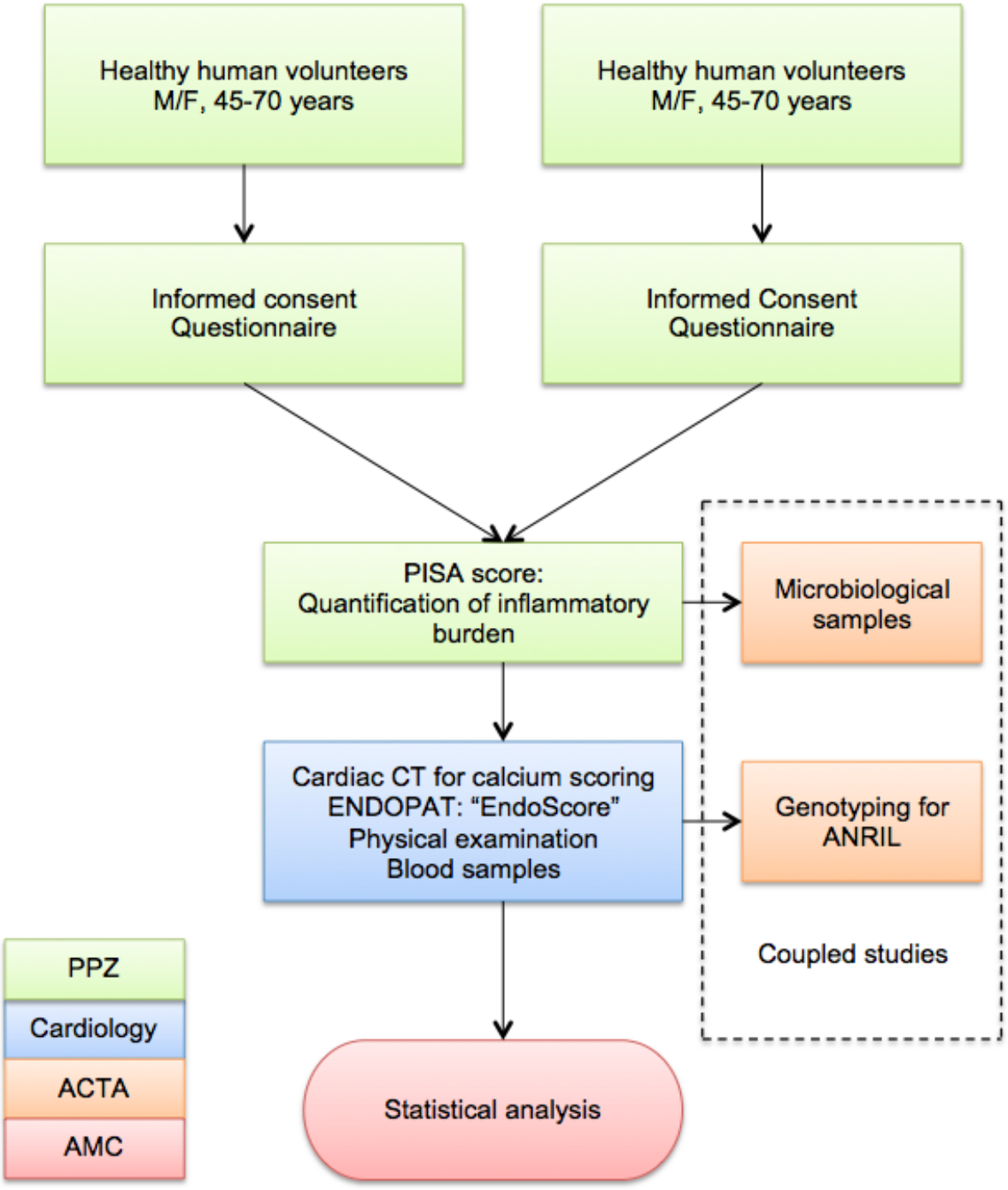
Periodontal examination
Location: PPZ
Procedures:

- Signing **informed consent**
- **Questionnaire** about medical and dental history, perceived health, parental history, lifestyle, socio-economic status and oral hygiene.
- **Periodontal examination**: periodontal probing pocket depth (PD), gingival recession, plaque score, and bleeding on probing (BOP) measurements. All measurements will be performed on all teeth, on six sites per tooth. The periodontal inflamed surface area (**PISA**) will be calculated.
- **Bacterial sample** (on behalf of the microbiome study)

Medical examniation
Location: Department of cardiology ISALA
Procedures:

- **Physical examination**: Blood Pressure (BP), Heart Rate (HR), Body Mass Index (BMI), Waist to Hip ratio (WHR), Electrocardiogram (ECG)
- **Blood sample**: HsCRP, total cholesterol, HDL, LDL, triglycerides, eGFR, HbA1c
- **ENDOPAT**: Endothelial function (“EndoScore”)
- Extra blood sample for DNA extraction and genotyping (on behalf of the common gene analyses for both diseases)
- **Cardiac Computed Tomography (CCT)** for Coronary Calcium Scoring (CCS)

7.4 Flow chart study procedures



7.5 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

7.6 Replacement of individual subjects after withdrawal

We will not replace individual patients after withdrawal. As this is a cross-sectional pilot study, we will recruit until 50 subjects have been investigated.

7.7 Follow-up of subjects withdrawn from treatment

The reason for withdrawal of each patient will be recorded: further treatment and policy will be performed by the treating dentist and physician.

7.8 Premature termination of the study

Not applicable for this study.

8. SAFETY REPORTING

8.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

8.2 Adverse and serious adverse events

If adverse events are encountered, the treating physician is responsible for adequate follow-up. The Practice for Periodontology Zwolle (PPZ) is responsible for the periodontal examination and periodontal treatment.

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / the experimental treatment]. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

A serious adverse event is any untoward medical occurrence or effect that at any dose:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- is a new event of the trial likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction, lack of efficacy of an IMP used for the treatment of a life threatening disease, major safety finding from a newly completed animal study, etc.

All SAEs will be reported through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse reactions..

SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse reaction. This is for a preliminary report with another 8 days for completion of the report.

8.2.1 Suspected unexpected serious adverse reactions (SUSAR)

Not applicable for this study.

8.2.2 Annual safety report

Not applicable for this study.

8.3 Follow-up of adverse events

All adverse events will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

8.4 Data Safety Monitoring Board (DSMB)

Not applicable for this study.

9. STATISTICAL ANALYSIS

Primary outcome is the correlation between the PISA score and the coronary calcium score. Therefore 2 groups of 25 patients will be analysed. One group diagnosed with periodontal disease and one group with a healthy periodontium. For description of the data the mean values, standard deviations, minimum and maximum are calculated. For continuous data, differences between the groups will be analysed using the independent T-test. If the data violates the assumption of a normal distribution, differences between the groups will be analysed using the Mann-Whitney test. Binary data will be assessed using the Pearson chi-square test. Correlation between the overall PISA score and the coronary calcium score will be calculated with the Spearman correlation test. Outcome variables that show significant between-groups differences at baseline will be used as covariate in a multivariate regression analysis. Data will be entered in a database (SPSS) and analyzed using SPSS statistical package 18.0 (SPSS Inc. Chicago, Il

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (June 1964, as modified by the 56th World Medical Association, October 2008), and in accordance with the Medical Research Involving Human Subjects Act (WMO)

10.2 Recruitment and consent

The responsible physician/ dentist will introduce the study to the patients. If a patient meets the inclusion criteria, they will be fully informed about the study by the investigator (through personal contacts and written information brochures). The decision regarding participation in the study, which will be made by the patient, is entirely voluntary. Refusal of participation

won't have any consequences for further treatment of the patient. Written informed consent to the study will be obtained at the first day of measurement.
(See appendix for patient information and informed consent)

10.3 Benefits and risks assessment, group relatedness

Early diagnosis of atherosclerosis, coupled with awareness and possible disease modifying treatment, including diagnosis of periodontitis, has been shown to have a favourable effect on the course of the disease.

10.4 Compensation for injury

An insurance that falls within the scope of the WMO is available in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 450.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 3.500.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
3. € 5.000.000,-- (i.e. five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.5 Incentives

Participants will only receive their travel costs. Participants will not receive any special incentives, compensation or treatment through participation in this study.

11. ADMINISTRATIVE ASPECTS AND PUBLICATION

11.1 Handling and storage of data and documents

A subject's identification code list will be used to link the data to the subjects. The code consists of two letters followed by increasing numbers beginning with 001. The investigator will safeguard the key to the code. Data may be restored for a longer period then necessary for the relevant investigation. This will be at max. 15 years. This storage and every eventual renewed use occur however in agreement with the regulations of this Code od Conduct, in particular article 5.5 and chapter 7 of FMWV Code of Conduct for health research. Personal data obtained according to the regulations of this chapter may only be saved insofar as it is reasonable to expect that they may be needed for the investigation later. The handling of personal data complies with the Dutch personal Data Protection Act.

11.2 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion. All substantial amendments will be notified to the METC and to the competent authority. Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

11.3 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject,

numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.4 End of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the moment when the analyses are done and the research questions are answered.
In case the study is ended prematurely, the investigator will notify the accredited METC, including the reasons for the premature termination.
Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

11.5 Public disclosure and publication policy

The results of our study will be disclosed and published unreservedly.

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