PROTOCOL TITLE

'*Periodontitis as a potential early risk indicator for diabetes mellitus, atherosclerotic cardiovascular disease, metabolic syndrome and obstructive sleep apnea syndrome*'

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Pharmacy	***Not applicable***

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

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)**
ACTA	**Academic Centre for Dentistry Amsterdam**
ADA	**American Diabetes Association**
ASCVD	**Atherosclerotic Cardiovascular Disease**
BMI	**Body Mass Index**
CDC-AAP	**Centers for Disease Control and Prevention**
DM	**Diabetes Mellitus**
ESC	**European Society of Cardiology**
HbA1c	**Glycated Haemoglobin**
HDL-C	**High-density Lipoprotein Cholesterol**
IC	**Informed Consent**
IDF	**International Diabetes Federation**
LDL-C	**Low-density Lipoprotein Cholesterol**
MetS	**Metabolic Syndrome**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
NHG	**Nederlands Huisartsen Genootschap (Dutch Family Physicians Clinical Practical Guidelines)**
NCEP ATP III	**National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III)**
OSA	**Obstructive Sleep Apnea**
OSAS	**Obstructive Sleep Apnea Syndrome**
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.**
STROBE	**Strengthening the Reporting of Observational studies in Epidemiology**
WC	**Waist Circumference**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Diabetes Mellitus (DM), Atherosclerotic Cardiovascular Disease (ASCVD), Metabolic Syndrome (MetS) and Obstructive Sleep Apnea Syndrome (OSAS) are major health problems (1-3). However due to absence of symptoms and/or lack of knowledge, people are often unaware of having DM, ASCVD, MetS or OSAS while early treatment could prevent the progression of these diseases. Therefore, risk indicators for early detection of DM, ASCVD, MetS and OSAS are needed and proposed (4-8).

Many studies demonstrate the association between oral diseases and DM and/or ASCVD (9, 10). The most observed chronic oral disease is periodontitis. Periodontitis is a common chronic multifactorial inflammatory disease of the supporting structures of the teeth (11). DM and ASCVD might, due to their pro-inflammatory state and alteration of the immune system, contribute to the onset and/or progression of periodontitis (12). Therefore periodontitis could be considered as an early sign of an underlying vascular and/or metabolic pathology (13).

Some studies have also reported a positive association between periodontitis and MetS (14). The MetS prevalence in patients with periodontitis was shown to be higher than controls and MetS showed to be associated with severe periodontitis (15, 16). The association between periodontitis and OSAS is less established, however there is some evidence to a plausible association between periodontal disease and Obstructive Sleep Apnea (OSA) (8).

Due to the broad overlap of objective biomarkers, in this study, subjects with or without periodontitis will be screened for determining the prevalence of (pre)DM, the 10-year-risk of ASCVD, the prevalence of MetS and the risk of OSAS. The screening will be based on clinical measures, specific blood markers determined by a developed finger stick analysis and three questionnaires.

Notably, a recently published pilot paper from our research group presents the feasibility and preliminary results of proposed screening for (pre)DM; we found 18.1% new DM cases among subjects with severe periodontitis compared to 9.9% in mild/moderate periodontitis and 8.5% in controls (13).

Objective: The aim of the proposed study is to screen for (pre)DM, an increased 10-year-risk of ASCVD, MetS and a high risk of OSAS in subjects with and without periodontitis.

Study design: Cross-sectional study

Study population: In total 783 patients will be included (261 without periodontitis, 261 with mild/moderate periodontitis and 261 with severe periodontitis) at the Academic Centre for Dentistry Amsterdam (ACTA). The subjects with periodontitis that will be enrolled in this study are referred to the periodontal clinic for diagnosis and treatment of periodontitis. The subjects without periodontitis will be selected among individuals that visit the dental school for regular dental checkups. All subjects will be 18 years and older and are mainly inhabitants from Amsterdam and surrounding areas.

Intervention (if applicable): Subjects with and without periodontitis will undergo the following intervention:

- General, medical and dental histories (standard procedure)
- Clinical periodontal evaluation (standard procedure)
- Dental x-rays, when indicated for periodontitis or other dental pathology (standard procedure)
- Measurement of systolic and diastolic blood pressure (standard procedure)
- Measurement of length and body weight, waist and neck circumference, a questionnaire for demographic characteristics, an International Physical Activity Questionnaire (IPAQ) and an OSAS risk questionnaire (extra procedure)
- Plasma finger stick procedure (HemCol, Labonovum BV) for measuring biomarkers: A drop of blood is obtained with a finger stick (Greiner Bio-one Safety Lancet) and collected in a collection tube. The collection tube is sent to the laboratory for analysis by regular mail. (extra procedure)

Main study parameters/endpoints: To investigate, within a dental setting, the differences between subjects with and without periodontitis in:

- The prevalence of preDM and DM
- The 10-year-risk of ASCVD
- The prevalence of MetS
- The risk of OSAS

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

- The extra burden for the subjects with and without periodontitis will be three questionnaires, measurement of length, body weight, waist and neck circumference, and a finger stick procedure.
- The risks associated with participation are negligible.
- Possible benefit of participation will be early detection of health risks such as (pre)DM, ASCVD, MetS, OSAS and possibly a referral for further diagnosis and/or treatment at the family physician.

1. INTRODUCTION AND RATIONALE

Diabetes Mellitus (DM), Atherosclerotic Cardiovascular Disease (ASCVD), Metabolic Syndrome (MetS) and Obstructive Sleep Apnea Syndrome (OSAS) are major health problems (1-3). However due to the absence of symptoms and/or disease-related knowledge, the pathology of DM, ASCVD, MetS and OSAS often goes undetected. Approximately one-third of the people with DM are not aware of their status (17). Another study has shown that the onset of DM appears approximately 4 to 7 years before clinical diagnosis (18). Research in Dutch OSAS patients showed that in 66% of the patients it took ≥4 years before they were diagnosed with OSAS (4). Therefore, risk indicators for DM, ASCVD, MetS and OSAS are needed and proposed (4-8). In this respect the onset of several oral pathologies might be indicative.

Many studies demonstrate the association between oral diseases and DM and/or ASCVD (9, 10). The most observed chronic oral disease is periodontitis. For MetS and OSAS this association is less clear (8). However different studies have suggested an association between periodontitis and MetS (14-16) or between periodontitis and OSAS (8, 19). Periodontitis is a common chronic multifactorial inflammatory disease of the supporting structures of the teeth (root cementum, gingiva, periodontal ligament and alveolar bone). This condition is initiated and sustained by an aberrant host immune response against resident bacterial biofilms on the teeth and the tooth roots (11). Although the relative contribution of several causal factors may vary among patients, the uncontrolled inflammatory response upon bacterial accumulation seems the major factor determining the onset and/or progression of the periodontitis (12). In this respect, an increased pro-inflammatory state caused by processes like atherosclerosis and metabolic dysregulation might contribute to the onset and/or progression of periodontitis. Therefore periodontitis could be considered as an early sign of an underlying vascular and/or metabolic pathology (13). With this knowledge, it has been suggested that dentists could help to screen for DM, the risk of ASCVD, MetS and the risk of OSAS.

Notably, today there are several easy accessible and non-invasive techniques to screen for DM, ASCVD, MetS and OSAS. For DM there is the convenient Haemoglobin A1c (HbA1c) level, which is put forward by the American Diabetes Association (ADA) and International Diabetes Federation (IDF) as indicative of the metabolic state: prediabetes (HbA1c: 39-47 mmol/mol) and diabetes (HbA1c: ≥48 mmol/mol) (5,6). For ASCVD there is the “10-year-risk” chart, based on age, gender, total cholesterol, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), systolic and diastolic blood pressure, smoking, presence of diabetes and rheumatoid arthritis (according to the NHG standards, which are the European Society of Cardiology guidelines modified to the Dutch population)(20). The diagnostic criteria for MetS are based on waist circumference (WC), triglycerides (TG), HDL-C, hypertension and dysglycemia (according to the NCEP ATP III) (21). For OSAS there is the recently developed OSAS risk questionnaire, which scores patients into a low, moderate or high risk category of having OSAS (22).

The majority of dental practices is not equipped for blood chemistry, however, today all plasma markers can be measured easily and accurately with help of a finger stick procedure (HemCol, Labonovum B.V., Rotterdam, The Netherlands). The measurement of the HbA1c and lipid by finger stick procedure is an elegant and essentially non-invasive way to screen for DM, ASCVD and MetS biomarkers. In addition, other demographic parameters (such as age, gender, smoking and rheumatoid arthritis) are known from the medical history and obtained with a questionnaire. Also systolic and diastolic blood pressure, Body Mass Index (BMI), Waist Circumference and N (WC) will be measured. A dental office, and in particular the dental office with a high volume of periodontitis patients, could be a suitable location for screening of individuals for early signs of DM, ASCVD, MetS and OSAS.

The importance of early identification of subjects with increased risk for DM, ASCVD and MetS lies in the prevention of DM associated micro- and macrovascular complications, future coronary heart disease and/or cerebrovascular events. Early identification in OSAS patients would result in better medical and psychological conditions. Therefore, it contributes to an increased quality of life of our patients. The results of the current study should indicate the prevalence of DM and MetS and the prevalence of patients being at increased risk for ASCVD or OSAS. This may form the basis for further studies into general applicability and cost-effectiveness.

Hypothesis

- We put the hypothesis forward that periodontitis patients show an increased prevalence of (pre)DM, an increased 10-year-risk of ASCVD, an increased prevalence of MetS and an increased risk of OSAS compared with subjects without periodontitis.

Notably, a recently published pilot paper from our group presented the feasibility and preliminary results of proposed screening for (pre)DM; we found 18.1% new DM cases among subjects with severe periodontitis compared to 9.9% in mild/moderate periodontitis and 8.5% in controls (13).

2. OBJECTIVES

Primary Objective:

To investigate, within a dental setting, the differences between subjects with and without periodontitis in:

- The prevalence of preDM and DM (established cut-off values for HbA1c, appendix 1 and 2 (7))
- The 10-year-risk of ASCVD ("10-year-risk" chart, appendix 3 (20))
- The prevalence of MetS (diagnostic criteria of NCEP ATP III, appendix 4 (21))
- The risk of OSAS (OSAS risk questionnaire (22))

Secondary Objective(s):

- To investigate the differences in (pre)DM prevalence, 10-year-risk of ASCVD, MetS prevalence and OSAS risk between mild/moderate and severe periodontitis patients

3. STUDY DESIGN

A cross-sectional study will be performed with a duration of 24 months at the dental clinic of the Academic Centre for Dentistry Amsterdam (ACTA).

4. STUDY POPULATION

4.1 Population (base)

The research population will be drawn from the patients that visit the ACTA. These patients are particularly from Amsterdam and surrounding areas. The study subjects with periodontitis that will be enrolled in this study are referred to the periodontal clinic of ACTA for diagnosis and treatment of periodontitis. The control subjects without periodontitis will be selected among subjects that visit the dental school of ACTA for regular dental checkups.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Age ≥ 18 years
- (Patient) referred for periodontitis
- (Control) visiting the dental school for regular dental check-up without periodontitis
- Able and willing to give written informed consent and comply with the requirements of the study protocol.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- None

4.4 Sample size calculation

The calculation of the sample size was based on a Chi^2-test having an $\alpha<0.05$ with 90% power and using the prevalence of diabetes of two groups (without periodontitis: 8.5%, severe periodontitis: 18.1%)(13). The required sample size is a minimum of 261 subjects per group.

5. TREATMENT OF SUBJECTS

NA

5.1 Investigational product/treatment

NA

5.2 Use of co-intervention (if applicable)

NA

5.3 Escape medication (if applicable)

NA

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

NA

6.2 Summary of findings from non-clinical studies

NA

6.3 Summary of findings from clinical studies

NA

6.4 Summary of known and potential risks and benefits

NA

6.5 Description and justification of route of administration and dosage

NA

6.6 Dosages, dosage modifications and method of administration

NA

6.7 Preparation and labelling of Investigational Medicinal Product

NA

6.8 Drug accountability

NA

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

NA

7.2 Summary of findings from non-clinical studies

NA

7.3 Summary of findings from clinical studies

NA

7.4 Summary of known and potential risks and benefits

NA

7.5 Description and justification of route of administration and dosage

NA

7.6 Dosages, dosage modifications and method of administration

NA

7.7 Preparation and labelling of Non Investigational Medicinal Product

NA

7.8 Drug accountability

NA

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

To investigate, within a dental setting, the differences between subjects with and without periodontitis:
- The prevalence of preDM and DM (established cut-off values for HbA1c, appendix 1 and 2 (7))
- The 10-year-risk of ASCVD ("10-year-risk" chart, appendix 3 (20))
- The prevalence of MetS (diagnostic criteria of NCEP ATP III, appendix 4 (21))
- The risk of OSAS (OSAS risk questionnaire (22))

8.1.2 Secondary study parameters/endpoints (if applicable)

- To investigate the differences in (pre)DM prevalence, 10-year-risk of ASCVD, MetS prevalence and OSAS risk between mild/moderate and severe periodontitis patients.

8.1.3 Other study parameters (if applicable)

- Other study parameters that will be obtained are sex, age, ethnicity (origin of parents), education level, smoking habits, diagnosis of hypertension, diagnosis of ASCVD, medical treatment for ASCVD, relatives with ASCVD, diagnosis of hypercholesterolemia, medical treatment for hypercholesterolemia, diagnosis of rheumatoid arthritis, diagnosis of DM, relatives with DM, history of periodontal treatment, height and body weight (for determining the BMI), neck and waist circumference, blood pressure, physical activity.

8.2 Randomisation, blinding and treatment allocation

NA

8.3 Study procedures

All subjects with periodontitis, diagnosed in a periodontology clinic, will be asked to participate. Subjects without periodontitis, visiting the dental school for a dental check-up, are selected and also asked to participate. The selection of subjects without periodontitis will be based on the following criteria: no interproximal alveolar bone loss on ≤1-year-old dental bitewing radiographs, no fulfillment of criteria for the classification of periodontitis according to the Centers for Disease Control and Prevention – American Academy of Periodontology (CDC-AAP) case definition (Appendix 5) (23).

Medical information, a periodontal chart (for periodontitis patients) or a periodontal screening index (for subjects without periodontitis) and a standard dental radiographic chart will be retrieved from the patient records. During the dental appointment blood will be obtained by a finger stick. Blood analysis will be performed to acquire data on the condition of the patients.

A questionnaire about demographic characteristics will be filled in together with the periodontist or researcher, including the measurement of the systolic and diastolic blood pressure, body length and weight, waist and neck circumference. A questionnaire for assessment of the OSAS risk and a questionnaire about physical activity (IPAQ) will be asked to be filled in by the participant.

At the dental appointment, all subjects (with or without periodontitis) will undergo the following assessments during the periodontal intake or regular dental check-up:

1. General, medical and dental anamnesis (standard procedure)
2. Clinical periodontal evaluation to define the presence and severity of periodontitis (standard procedure)
3. Dental x-rays, when indicated for periodontitis or other dental pathology (standard procedure)
4. Measurement of systolic and diastolic blood pressure (standard procedure)
5. Measurement of length and body weight, waist and neck circumference, a questionnaire for demographic characteristics, an International Physical Activity Questionnaire (IPAQ) and an OSAS risk questionnaire. (extra procedure)
6. Consecutive plasma finger stick procedure (HemCol, Labonovum B.V.) for measuring biomarkers: HbA1c, total cholesterol, LDL-C, HDL-C, triglycerides, C-reactive protein (CRP), creatinine. A drop of blood is obtained with a finger stick (Greiner Bio-one Safety Lancet) and collected in a collection tube. The collection tube is sent to the laboratory for analysis by regular mail. (extra procedure)

Ad 5. The questionnaire for demographic characteristics contains a question about the ethnicity of the subject. The ethnicity is an important factor that is associated with DM and ASCVD. According to the NHG standards, the Turkish, Moroccan or Surinam-Hindustan origin is one of the risk factors for the screening of DM (24). Furthermore, it is known that ethnic differences have an influence on the HbA1c value. Factors such as differences in hemoglobin glycation, red cell survival or non-glycemic genetic determinants are being explored as determinants (25).

People of the Dutch population with a Turkish, Moroccan and Surinam-Hindustan origin have more often DM than people with a Dutch origin (26, 27). Also the prevalence of cardiovascular complications in diabetes patients is higher in people from these ethnic minorities, especially from Surinam-Hindustan. Cardiovascular diseases were more often diagnosed in people of Turkish origin compared to people of Dutch origin (28). Our study population in Amsterdam and surrounding areas has a

variety of different ethnic groups. Because ethnicity is one of the factors, related to DM and ASCVD, it has to be taken into account as a variable when measuring the biomarkers for DM and ASCVD.

All subjects will receive two letters with the result of the plasma finger stick procedure, on which the values of HbA1c, total cholesterol, LDL-C, HDL-C, triglycerides, CRP, creatinine will be reported. One letter is for the subject and the other letter is for their general practitioner (GP). The patient can decide to bring this letter to their GP. All subjects will be orally informed about their systolic and diastolic blood pressure by their dentist or researcher. When the blood values are above the known cut-off values and/or the 10-year risk of ASCVD is increased and/or the risk of OSAS is high, then the patient will be advised to visit the general practitioner for further diagnosis. An information brochure from the Thuisarts.nl, developed by the NHG, will be given when a high risk of cardiovascular diseases is suspected. Another information brochure from the Dutch Organization for Sleep Apnea about sleep apnea will be given when the risk of OSAS is increased.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. However, during informed consent, subjects will be informed that participation is not possible if they don't want to be informed about the results of the finger stick procedure or questionnaires. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal (if applicable)

NA

8.5 Replacement of individual subjects after withdrawal

NA

8.6 Follow-up of subjects withdrawn from treatment

NA

8.7 Premature termination of the study

NA

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the finger stick procedure. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

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

- 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; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events, except for the following SAEs: NA

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a

period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

NA

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product;
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

9.3 Annual safety report

NA

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.4 Follow-up of adverse events

All AEs 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. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

9.5 [Data Safety Monitoring Board (DSMB) / Safety Committee]

NA

The advice(s) of the DSMB will only be sent to the sponsor of the study. Should the sponsor decide not to fully implement the advice of the DSMB, the sponsor will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

10. STATISTICAL ANALYSIS

The prevalence of (pre)DM and MetS, the 10-year risk of ASCVD and the risk of OSAS will be presented in tables. First, one table will be generated describing the background variables, blood chemistry and demographic characteristics. Secondly, the prevalence of (pre)DM and MetS, the 10-year-risk of CVD and the OSAS risk will be reported in patients with and without periodontitis.

According to the ADA guidelines HbA1c values will be classified as normal (<5.7% [<39 mmol/mol]), preDM (5.7-6.4% [39-47 mmol/mol]) and DM (≥6.5% [≥48 mmol/mol]) (7).

The 10-year risk of ASCVD will be assessed with help of the risk table from the Dutch guideline on cardiovascular risk management (CVRM) according to the standards of the Dutch College of General Practitioners (NHG) (20).The NHG standards are derived from European Society of Cardiology guidelines and modified to the Dutch situation (6).

According to the NCEP ATP III guidelines MetS is present if three of the 3 or more of the five criteria are met: elevated waist circumference over ≥ 102 cm (men) or ≥ 88 cm (women), triglyceride (TG) level over ≥ 1.7 mmol/L or being on drug treatment for elevated TG, high density lipoprotein cholesterol (HDL-C) level less than < 1.03 mmol/L (men) or < 1.3 mmol/L (women), blood pressure over 130/85 mmHg or on antihypertensive drug treatment, and blood glucose level over ≥ 5/6 mmol/L (21).

The OSAS risk is calculated with help of a new questionnaire (PhilipsQ) which is constructed from body measurements (sex, age, BMI, neck circumference) and the most predictive questions of the following OSAS and sleep questionnaires: Berlin Questionnaire (BerlinQ), STOP Questionnaire (STOPQ) and Athens Insomnia Scale (AIS). An algorithm is developed to compute the individual estimated probability (p) that a subject will have OSAS: p(OSAS) = 1 / (1+e-X), where X = −6.322 + 2.828 × AIS + 2.745 × STOPBANGQ High + 0.965 × STOPBANGQ Intermediate + 4.640 × BerlinQ High + 1.584 × BerlinQ Intermediate + 0.790 × Age + 0.810 × BerlinQ Q-5. The final cutoff values were determined by exploratory logistic regression analyzes. The risk of OSAS will be classified as low (p[OSAS] < 35%), intermediate (p[OSAS] = 35-55%) and high (p[OSAS] > 55%) (22).

The missing data from included subjects will be imputed using the expectation-maximization method. Background variables, blood chemistry and demographic data between cases and controls will be analysed by Chi^2-analyses or t-tests where

applicable. The primary and secondary outcome variables will be analysed both by univariate analysis and multivariate analysis (multiple logistic regression). The data will be analysed on an intention-to-treat basis (ITT).

10.1 Primary study parameter(s)

The cases and controls are a priori defined by inclusion and exclusion criteria. The STROBE guideline will be followed and a flow diagram will be generated of how many individuals have been screened, invited to participate, included and analysed (29). All initially included study subjects will form part of the data analysis (intention to treat). Type I error will be controlled by including a sufficient number of cases and controls to avoid multiplicities and to apply Bonferroni corrections where applicable. Secondly, we have determined the minimal sample size on the basis of an appropriate power calculation (see chapter 4.4).

The prevalence of (pre)diabetes, the 10-year risk of ASCVD, the prevalence of MetS and the prevalences of OSAS risks (categorical variables) within the study population will be compared with parametric and non-parametric tests (ANOVA and Chi^2-test). Where applicable, analyses will be corrected for multiple testing (Bonferroni). The significance level will be set to $p<0.05$.

The prevalence of (pre)DM among cases and controls will be firstly reported without adjustment of any of potential covariates. In a second exploratory analysis we will adjust the prevalence for general and diabetes and ASCVD related characteristics of the study population: sex, age, ethnicity (origin of parents), education level, smoking habits, relatives with DM, history of periodontal treatment, diagnosis of hypertension, diagnosis of hypercholesterolemia, diagnosis of DM and the BMI, amount of physical activity.

The 10-year risk of ASCVD will be calculated according to the algorithm from the ESC guidelines, which is adapted to the Dutch situation. No further adjustments will be done.

The prevalence MetS will be calculated according to the criteria of the NCEP ATP III guidelines. No covariates will be included.

The risk for OSAS will be assessed by the OSAS questionnaire. No further adjustments will be done. However, the results will be explored in relation to having periodontitis, the

background characteristics, the blood chemistry outcomes and having (pre)diabetes and the 10-year risk for ASCVD.

10.2 Secondary study parameter(s)

The secondary research aim is to investigate whether there are differences between severe periodontitis and mild/moderate periodontitis. As such, mild/moderate periodontitis will be labelled as “control” while severe periodontitis patients are labelled as “cases”. In secondary analyses exact procedures are followed as described above for the primary outcomes.

10.3 Other study parameters

Other study parameters that will be obtained and used as covariates are sex (M/F), age (years), ethnicity (origin of parents) (Europe/Non-Europe), education level (high/low), smoking habits (yes/no), diagnosis of hypertension (yes/no) , diagnosis of ASCVD (yes/no), medical treatment for ASCVD (yes/no), relatives with ASCVD (yes/no), diagnosis of hypercholesterolemia (yes/no), medical treatment for hypercholesterolemia (yes/no), diagnosis of rheumatoid arthritis (yes/no), diagnosis of DM (yes/no), relatives with DM (yes/no), history of periodontal treatment (yes/no), height (cm) and body weight (kg) (for determining the BMI), neck and waist circumference (cm), blood pressure (mmHg), physical activity (Metabolic Equivalent of Task).

10.4 Interim analysis (if applicable)

NA

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The procedures set out in this study protocol are designed to ensure that the investigator abide by the principles of the good clinical practice guidelines of the European Community and the Declaration of Helsinki (19-10-2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) in the conduct, evaluation and documentation of this study. The study will also be carried out in keeping with local legal requirements.

11.2 Recruitment and consent

Patients with periodontitis

Patients with periodontitis, referred to the department of Periodontology (ACTA), will receive an information letter about the study from their dental practitioner (of ACTA) at least 1 week before their visit to the periodontal clinic. The dental practitioner is informed by the researcher about the study and the recruitment of patients. During the appointment, patients will be asked by the dental practitioner whether they want to participate with the study. Patients can give consent. The terms of the consent and when it was obtained will be documented in the case record form. After the informed consent, the dental practitioner or researcher will fill in the questionnaires with the patients and use the finger stick analysis.

Control subjects

Patients, visiting the dental clinic at ACTA for a regular dental checkup, will receive an information letter about the study from their dental practitioner (of ACTA) at least 1 week before their visit to the dental clinic. The dental practitioner is informed by the researcher about the study and the recruitment of patients. The dental practitioner performs the dental checkup. If the patient meets the inclusion criteria (see 8.3 Study procedures), then he/she will be asked by the dental practitioner (of ACTA) whether he/she wants to participate with the study. Patients can give consent. The terms of the consent and when it was obtained will be documented in the case record form. After the informed consent, the researcher will fill in the questionnaires with the patients and use the finger stick analysis.

Before being admitted to the clinical study, the patient must consent to participate after the nature, scope and possible consequences of the clinical study have been explained in an information letter understandable to him/her.

In addition to the standard requirements that physicians are currently obliged to observe when providing information, all points on the information letter must be covered. The investigator will not undertake any investigation specifically required only for the clinical study until valid consent has been obtained. If the patient does not wish to know the result of the finger stick procedure, then he/she will not be included in the study.

11.3 Objection by minors or incapacitated subjects (if applicable)

NA

11.4 Benefits and risks assessment, group relatedness

- The extra burden for the patients and control subjects will be three questionnaires, measurement of length, body weight, waist and neck circumference, and a finger stick procedure.
- The risks associated with participation are negligible.
- Possible benefit of participation will be early detection of health risks such as (pre)DM, ASCVD, MetS, OSAS and possibly a referral for further diagnosis and/or treatment at the family physician.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 650.000,-- (i.e. six 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 each research;
3. € 7.500.000,-- (i.e. seven million five hundred thousand Euro) each year for all researches together.

All participants of this study will be informed about the insurance.

11.6 Incentives (if applicable)

NA

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

All patient names will be kept secret. The patient number during the study will identify patients throughout documentation and evaluation. At inclusion, each study subject receives a research number. Each questionnaire is coded with a patient number and a research number. The research number corresponds to the blood sample of the finger stick procedure. The tubes with the obtained blood will be sent to the chemical laboratory of Labonovum B.V. with the coded research number written on it. This laboratory will report the results of the finger stick procedure for each research number. This way the laboratory will not have any insight to individual data of our patients.

The patients will be told that all study findings will be stored on computer and handled in strictest confidence. The investigator also agrees to allow these to be inspected on request. The investigator will maintain a personal list of patient numbers and patient names to enable records to be found at a later date. This personal list will be kept physically apart from the other data. The result of the finger stick procedure will be added to the medical history of the patient. Other documents and results, with individual patient numbers, will be kept for 15 years.

12.2 Monitoring and Quality Assurance

An independent monitor (quality officer) will monitor the study data according to Good Clinical Practice (GCP). During the selection of the study subjects, Informed Consents will be checked. In addition, Source Data Verification will be performed during onsite monitoring (checking whether the data of the Case Report Forms (questionnaires) are similar to the source data (patient status, lab results etc.). The intensity of this verification is related to the risk of the investigation. Data that also will be checked are the inclusion and exclusion criteria and the primary outcomes of the research. The monitor will also check whether all (S)AE's and SUSAR's are reported adequately within the time schedules as required by the law or regulations.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. The amendments will be submitted to the METC. All amendments will be notified to the METC that gave a favourable opinion. The starting date of the study will also be informed to the METC.

NA

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

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.

12.4 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.

12.5 Temporary halt and (prematurely) end of study report

<*The following text is applicable for studies <u>without</u> an investigational medicinal product*.>
The investigator/sponsor 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 last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, 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.

<The following text is applicable for studies with an investigational medicinal product.>

NA

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, 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 and the Competent Authority.

12.6 Public disclosure and publication policy

The Consortium Agreement following the CCMO statement regarding publication policy of the study results is subscribed.

8.9. Publication. Pursuant to the publication obligations set out in Section 5.12 of the TKI-Allowance Agreement, the Parties must ensure open access (free of charge, online access for any user) to all scientific publications relating to its Foreground under the Project subject to the conditions hereunder. In particular, the Parties shall ensure open access to the deposited publication at the latest: (i) on publication, if an electronic version is available for free via the publisher, or (ii) within six months of publication in any other case. A Party or Parties that intend to publish on the Foreground (jointly) owned by it shall provide the other Parties with the draft publication at least 30 (thirty) calendar days before publication. Any objection to the planned publication shall be made in writing to the Coordinator and the Party or Parties proposing the publication within 30 (thirty) calendar days upon receipt of the draft publication. If no objection is made within the time limit stated above, the Publication is permitted. For the avoidance of doubt, a Party shall not Publish Foreground or Background of another Party, even if such Foreground or Background is amalgamated with the Party's own Foreground, without the other Party's prior written approval.

8.10. Objections to Publication. An objection has to include a precise request for necessary modifications and shall be considered justified only, if:

a. the proposed publication includes another Party's Background, Foreground or other Confidential Information; or
b. the objecting Party's legitimate academic or commercial interests are harmed by the publication;
c. the proposed publication includes patentable Foreground and the objecting Party anticipates that it wishes to exercise the Option.
Upon receipt of an objection, the Parties involved shall discuss a solution in good faith. The objecting Party can request a publication delay of an additional period of 60 (sixty) calendar days (following the 30 day period referred to in clause 8.9). Upon expiration of the term, the publishing Party will be entitled to publish the proposed publication.

13. STRUCTURED RISK ANALYSIS

NA

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action
NA

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism
NA

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?
NA

d. Selectivity of the mechanism to target tissue in animals and/or human beings
NA

e. Analysis of potential effect
NA

f. Pharmacokinetic considerations
NA

g. Study population
NA

h. Interaction with other products
NA

i. Predictability of effect
NA

j. Can effects be managed?
NA

13.2 Synthesis

NA

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APPENDIX 1: Criteria for the diagnosis of DM according the ADA guidelines (7)

A1C ≥6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*
OR
FPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.*
OR
2-h plasma glucose ≥200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).

*In the absence of unequivocal hyperglycemia, result should be confirmed by repeat testing.

APPENDIX 2: Categories for pre-DM according the ADA guidelines (7)

FPG 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L) (IFG)
OR
2-h plasma glucose in the 75-g OGTT 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L) (IGT)
OR
A1C 5.7–6.4%

*For all three tests, risk is continuous, extending below the lower limit of the range and becoming disproportionately greater at higher ends of the range.

APPENDIX 3: The 10-year-risk chart of ASCVD (for patients without known ASCVD) (30)

Tabel 1. Risicotabel: 10-jaarsrisico op ziekte of sterfte door HVZ voor patiënten zonder HVZ

	Vrouwen										Mannen									
SBD	Niet-rookster					Rookster					Leef-tijd	Niet-roker					Roker			
180	35	38	41	43	44	47	50	>50	>50	>50		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
160	28	31	33	35	36	38	41	44	46	48		45	48	>50	>50	>50	>50	>50	>50	>50	>50
140	22	24	26	28	29	31	33	36	38	39	70	37	40	42	44	46	49	>50	>50	>50	>50
120	18	19	21	22	23	25	27	29	30	32		30	32	34	36	38	40	43	45	48	50
180	14	17	20	24	30	27	32	37	45	>50		25	30	36	44	>50	45	>50	>50	>50	>50
160	10	12	14	17	21	19	22	27	32	39		18	21	26	32	40	33	39	47	>50	>50
140	7	8	10	12	15	14	16	19	23	28	65	12	15	18	23	29	23	28	34	42	>50
120	5	6	7	9	11	10	11	14	17	20		9	11	13	16	21	17	20	24	30	38
180	10	12	15	18	23	20	23	28	34	42		22	26	32	40	50	40	48	>50	>50	>50
160	7	8	11	13	16	14	17	20	24	30		15	19	23	29	36	29	35	42	>50	>50
140	5	6	7	9	12	10	12	14	17	21	60	11	13	16	20	26	20	25	30	38	47
120	4	4	5	7	8	7	8	10	12	15		8	9	12	15	19	14	18	22	27	34
180	5	6	8	10	12	10	12	15	18	22		13	16	20	26	32	25	31	38	47	>50
160	4	4	5	7	9	7	8	10	13	16		10	12	15	18	23	18	22	27	34	43
140	3	3	4	5	6	5	6	7	9	11	55	7	8	10	13	17	13	16	19	24	31
120	2	2	3	3	4	4	4	5	6	8		5	6	7	9	12	9	11	14	17	22
180	2	3	4	5	6	5	6	7	9	11		8	10	12	15	20	15	18	23	28	36
160	2	3	3	3	4	3	4	5	6	8		6	7	9	11	14	11	13	16	20	26
140	1	1	2	2	3	2	3	3	4	6	50	4	5	6	8	10	7	9	12	15	19
120	1	1	1	2	2	2	2	2	3	4		3	3	4	6	7	5	7	8	10	13
180	1	1	1	1	1	1	1	1	2	2		3	3	4	6	7	5	6	8	10	13
160	<1	<1	1	1	1	1	1	1	1	2		2	2	3	4	5	4	4	6	7	9
140	<1	<1	<1	1	1	<1	<1	1	1	1	40	1	2	2	3	4	3	3	4	5	7
120	<1	<1	<1	<1	<1	<1	<1	1	1	1		1	1	2	2	3	2	2	3	4	5
	4	5	6	7	8	4	5	6	7	8		4	5	6	7	8	4	5	6	7	8
	Ratio totaal cholesterol/HDL											Ratio totaal cholesterol/HDL									

< 10% risico op ziekte of sterfte door HVZ; leefstijladviezen indien daar aanleiding voor is, zelden medicamenteuze behandeling.

10% tot 20% risico op ziekte of sterfte door HVZ; leefstijladviezen, medicamenteuze behandeling alleen bij risicoverhogende factoren en SBD > 140 mmHg en/of LDL > 2,5 mmol/l.

≥ 20% risico op ziekte of sterfte door HVZ; leefstijladviezen, medicamenteuze behandeling als SBD > 140 mmHg en/of LDL > 2,5 mmol/l.

Het risico bij patiënten met DM of RA kan worden geschat door bij de actuele leeftijd van de patiënt 15 jaar op te tellen.

APPENDIX 4: Criteria for diagnosis of Metabolic Syndrome (MetS) according to the NCEP ATP III (21)

Measure (any 3 of 5 constitute diagnosis of metabolic syndrome)	**Categorical cutpoints**
Elevated waist circumference (WC)	≥ 102 cm in men ≥ 88 cm in women
Elevated triglycerides (TG)	≥ 1.7 mmol/L or On drug treatment for elevated TG
Reduced HDL-C	< 1.03 mmol/L in men < 1.3 mmol/L in women or On drug treatment for reduced HDL-C
Elevated blood pressure	≥ 130 mmHg systolic blood pressure or ≥ 85 mmHg diastolic blood pressure or On antihypertensive drug treatment in a patient with a history of hypertension
Elevated fasting glucose	≥ 5.6 mmol/L

APPENDIX 5 Case Definitions Periodontitis according to the CDC-AAP (23)

Case	Definition†
No periodontitis	No evidence of mild, moderate, or severe periodontitis
Mild periodontitis	≥2 interproximal sites with AL ≥3 mm, and ≥2 interproximal sites with PD ≥4 mm (not on same tooth) or one site with PD ≥5 mm
Moderate periodontitis	≥2 interproximal sites with AL ≥4 mm (not on same tooth), or ≥2 interproximal sites with PD ≥5 mm (not on same tooth)
Severe periodontitis	≥2 interproximal sites with AL ≥6 mm (not on same tooth) and ≥1 interproximal site with PD ≥5 mm

* These definitions are now commonly referred to as the CDC–AAP case definitions for surveillance of periodontitis.

† Third molars excluded; total periodontitis is defined as the sum of mild, moderate, and severe disease.