

ORIGINAL RESEARCH/STUDY DESIGN

Periodontal Disease, Inflammation and Atherosclerosis Progression in Patients with Acute Coronary Syndromes – the ATHERODENT Study

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ABSTRACT

Recent studies have shown that systemic inflammation caused by periodontal disease (PD) can determine important changes in the coronary arteries, favoring atherosclerosis progression and the development of acute coronary syndromes (ACS). The **aim** of the ATHERODENT study (Protocol Record Number CM0117-ATD) is to assess the interrelation between PD, inflammation, and the progression of coronary atherosclerosis in patients with ACS. **Material and methods:** This case-control observational study will enroll 100 patients (group 1 – ACS and associated PD, and group 2 – ACS and no PD), in whom the following data will be collected: (1) demographic and clinical data; (2) cardiovascular risk factors; (3) full characterization of PD markers; (4) systemic inflammatory biomarkers; (5) imaging biomarkers derived from transthoracic echocardiography, computed tomography, coronary angiography, optical coherence tomography, and intravascular ultrasound; and (6) assessment of the presence of specific oral bacteria in samples of coronary plaques collected by coronary atherectomy, which will be performed during percutaneous revascularization interventions, when indicated in selected cases, in the atherectomy sub-study. The follow-up will be performed at 1, 3, 6, 12, 15, 18, and 24 months. The primary endpoint of the study will be represented by the rate of major adverse cardiovascular events (MACE) in PD vs. non-PD patients and in correlation with: (1) the level of systemic inflammation triggered by PD and/or by ACS at baseline; (2) the vulnerability degree of atheromatous plaques in the coronary tree (culprit and non-culprit lesions); and (3) the presence and burden of oral bacteria in atheromatous plaques. Secondary endpoints will be represented by: (1) the rate of progression of vulnerability degree of non-culprit coronary plaques; (2) the rate of progression of atheromatous burden and calcium scoring of the coronary tree; and (3) the rate of occurrence of left ventricular remodeling and post-infarction heart failure. The ATHERODENT study has been registered in clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT03395041).

Keywords: systemic inflammatory disease, periodontal disease, acute coronary syndromes, atherectomy

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STUDY RATIONALE

Coronary artery disease (CAD) and its major forms of manifestation — stable angina and acute coronary syndromes (ACS), are the most common causes of morbidity and mortality worldwide.¹ In 2015, the total number of cardiovascular deaths was 17.7 million, representing 31% of all deaths. From the total number of cardiovascular deaths, almost 50% (7.4 million) are caused by CAD.²

The underlying substrate of CAD is represented by atherosclerosis, a generalized chronic and progressive inflammatory disease determined by lipid accumulation in the sub-intimal arterial layer, which leads to progressive narrowing or blockage of coronary vessels.^{3,4} This process implies a proliferation of the smooth vascular cells due to invasion and accumulation of the macrophages at this site.⁵ Several pro-inflammatory mediators and cytokines (Interleukin-1, Interleukin-6, lymphocytes, alpha-tumor necrosis factor, and growth factors) contribute to the initiation of atheromatous plaque formation, favoring its progression and raising the risk of plaque rupture as a result of an increased plaque vulnerability.^{6,7} These mediators alter the structure of the endothelium, leading to overexpression of several adhesion molecules such as VCAM and ICAM, which enable leucocytes to bind to the endothelium.⁴

Epidemiologic studies identified various risk factors for CAD such as genetic factors, hypercholesterolemia, diabetes mellitus, smoking, or glucose intolerance. However, they are not specific for cardiovascular disease, being also associated with relevant triggers for other diseases such as periodontal disease (PD).⁸

PD is a chronic inflammatory disease of the tissues supporting the teeth, resulting from the activity of oral bacteria organized on the surface of the teeth. In PD, chronic inflammation extends from the periodontal pockets into the tooth-supporting tissue and leads to tooth loss.^{4,8} The prevalence of this disease is about 20–50% of global population in developed and developing countries.⁹ The microorganisms related with this disease are usually anaerobic Gram negative species or spirochetes. More than 800 species of bacteria have been identified in the oral cavity, the most prevalent ones found in sub-gingival tissues being represented by *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, and *Treponema denticola*. It has been demonstrated that these pathogenic agents can modify the activity of inflammatory cytokines and chemokines and alter the immune response of the host.^{4,9,10}

Several studies showed that inflammation represents the common link between PD and CAD. At the same

time, invasion of the coronary wall by species from the oral microbiota may trigger and accelerate the atherosclerotic process. The most frequently used inflammatory biomarker in clinical settings is represented by the highly-sensitive C-Reactive protein (hs-CRP), a protein which is known to be associated with cardiovascular risk and correlates with cardiovascular mortality.¹¹ Therefore, this biomarker can bring additional prognostic data on cardiovascular risk and may serve as an important clinical tool for risk stratification in patients with CAD.¹² The levels of hs-CRP correlate well with the severity of the atherosclerotic process, being involved in two inflammatory pathways: (1) local production by atheroma and vascular smooth cells and (2) cytokine-mediated production.¹³ At the same time, it is well known that one of the most important sites of CRP production is the human gingiva.⁴

In patients with CAD, PD may complicate the natural evolution of the atherosclerotic process and may increase the risk for plaque instability. A vulnerable plaque is a coronary plaque that is particularly exposed to an increased risk of plaque rupture, which triggers an ACS. A vulnerable plaque carries inside several markers of an increased vulnerability, which can be easily identified with the help of modern imaging techniques — the so-called “signatures of vulnerable plaque”, such as low-density atheroma, positive remodeling, spotty calcium, the presence of the necrotic core, enclosed by a thin fibrous cap depleted by inflammatory cells, or the napkin-ring sign.¹⁴ Newer intracoronary imaging techniques, such as optical coherence tomography (OCT), intravascular ultrasound (IVUS), thermography, spectroscopy, electron beam computed tomography, magnetic resonance, or multislice spiral computed tomography are extremely useful for detection of vulnerability markers showing an increased risk for developing an ACS.¹⁵

STUDY OBJECTIVES

The **primary objective** of ATHERODENT is to assess the interrelation between PD, inflammation, and atherosclerosis progression in patients who suffered an ACS and have concomitant PD vs. those with ACS and no PD, using (1) invasive and noninvasive imaging techniques for the characterization of vulnerable coronary plaques; (2) full characterization of PD; and (3) complex assessment of systemic vulnerability based on systemic inflammation-related biomarkers.

The **secondary objectives** of ATHERODENT are:

1. to study the correlation between PD and coronary plaque vulnerability;

TABLE 1. Protocol summary

Title	Periodontal Disease, Inflammation and Atherosclerosis Progression in Patients with Acute Coronary Syndromes – the ATHERODENT Study
Objectives	<p>Primary:</p> <ol style="list-style-type: none"> 1. To assess the interrelation between PD, inflammation, and atherosclerosis progression in patients who suffered an ACS and have concomitant PD vs. those with ACS and no PD, using (1) invasive and noninvasive imaging techniques for the characterization of vulnerable coronary plaques; (2) full characterization of PD; and (3) complex assessment of systemic vulnerability based on systemic inflammation-related biomarkers. <p>Secondary:</p> <ol style="list-style-type: none"> 1. To study the correlation between PD and coronary plaque vulnerability; 2. To assess the correlation between PD and severity of coronary atherosclerosis.
Study design	ATHERODENT is a case-controlled observational clinical study.
Study population	<p>The study will include 100 patients with ACS (unstable angina or NSTEMI-type acute myocardial infarction), who undergo invasive coronary angiography ± interventional revascularization according to local protocols, divided in 2 groups:</p> <ul style="list-style-type: none"> – Group 1 – patients with ACS in whom dental examination performed in the first 7 days after the index event revealed the presence of PD; – Group 2 – patients with ACS in whom dental examination performed in the first 7 days after the index event did not reveal the presence of PD. <p>Inclusion criteria:</p> <ul style="list-style-type: none"> – patients aged at least 18 years; – patients who have signed the written informed consent; – patients with ACS (unstable angina or non-ST segment elevation myocardial infarction) occurring with maximum 7 days prior to enrollment. <p>Exclusion criteria:</p> <ul style="list-style-type: none"> – patient's refusal to participate in the study; – sensitivity to the contrast substance; – women of reproductive age who do not use contraceptive methods; – pregnant women; – any malignancy within the last 5 years; – any disease or comorbidity that can reduce life expectancy to less than 2 years; – acute or chronic renal failure; – non-compliant patients who, in the opinion of the investigators, will not present to follow-up.
Study Duration:	01.01.2018 – 01.01.2021
Ethics:	<p>Ethical approval for this study has been obtained from both institutional boards – approval no. 25/28.12.2017 from the Ethics Committee of the Cardio Med Medical Center, and approval no. 351/13.12. 2017 from the University of Medicine and Pharmacy of Tîrgu Mureş.</p>

2. to assess the correlation between PD and the severity of coronary atherosclerosis;
3. to assess the presence and burden of oral bacteria in coronary atheromatous plaques collected during atherectomy and their relation with plaque vulnerability and evolution following an ACS (in the atherectomy sub-study).

The protocol summary of the ATHERODENT study is presented in Table 1.

STUDY DESIGN

ATHERODENT is a case-controlled observational clinical study, conducted in two clinical sites: the University of Medicine and Pharmacy of Tîrgu Mureş, Romania, and the Center of Advanced Research in Multimodality Cardiac Imaging of the Cardio Med Medical Center, Tîrgu Mureş, Romania (Protocol Record Number CM0117-ATD).

The ATHERODENT study has been registered in clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT03395041).

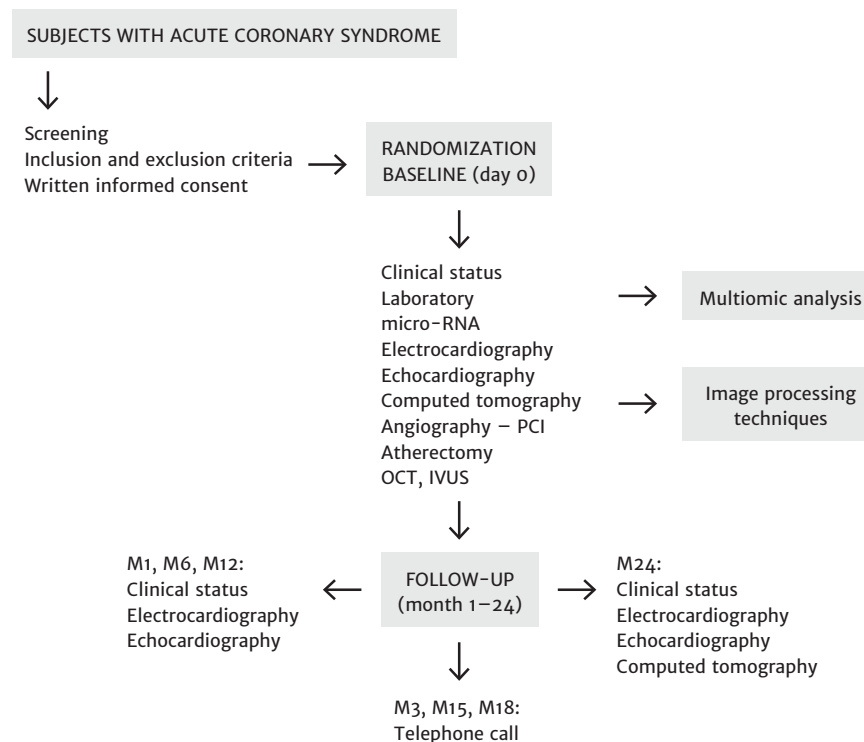


FIGURE 1. ATHERODENT study design

Baseline will be considered as the moment of the index event and related hospitalization. The index event will be considered the ACS, and patients will be randomized in the study at maximum 7 days post-ACS. The follow-up visits will be performed at 1, 3, 6, 12, 15, 18, and 24 months after randomization. Figure 1 represents a diagram of the overall study design.

GROUPS AND INTERVENTIONS

The study lot will consist of 100 patients with ACS (unstable angina or NSTEMI-type acute myocardial infarction), who undergo invasive coronary angiography ± interventional revascularization according to local protocols, divided in 2 groups:

- group 1 – patients with ACS in whom dental examination performed in the first 7 days after the index event revealed the presence of PD;
- group 2 – patients with ACS in whom dental examination performed in the first 7 days after the index event did not reveal the presence of PD.

Inclusion criteria:

- patients aged at least 18 years;

- patients who have signed the written informed consent;
- patients with ACS (unstable angina or non-ST segment elevation myocardial infarction) occurring with maximum 7 days prior to enrollment.

Exclusion criteria:

- patient's refusal to participate in the study;
- sensitivity to the contrast substance;
- women of reproductive age who do not use contraceptive methods;
- pregnant women;
- any malignancy within the last 5 years;
- any disease or comorbidity that can reduce life expectancy to less than 2 years;
- acute or chronic renal failure;
- non-compliant patients who, in the opinion of the investigators, will not present to follow-up.

STUDY PROCEDURES

The following procedures will be performed at baseline:

1. recording of demographic and clinical data (age, gender, personal history);

TABLE 2. Study procedures

	Baseline	Discharge	M1	M3	M6	M12	M15	M18	M24
Informed consent									
Inclusion/exclusion criteria									
Demographic data									
Clinical status									
Vital signs (HR, BP, SaO ₂)									
Weight									
Height									
Laboratory									
Micro RNA									
Matrix metalloproteinases determination									
Determination of bacterial antigens in the dental and coronary plaque									
ECG									
Echocardiography									
Angiography									
CT									
Atherectomy									
Determination of miRNA from the atheromatous plaque obtained by atherectomy									
OCT									
IVUS									
Microscopic analysis of tissue (atheroma)									
Endpoints									

- determination of serum lipids, blood counts, glycaemia, urea, creatinine, liver enzymes;
- determination of biomarkers expressing the severity of the acute coronary syndrome and heart damage (hs-Troponin, NT-proBNP);
- determination of serum levels of inflammatory biomarkers and adhesion molecules at the moment of the index event (hs-CRP, matrix metalloprotease, interleukin-6, VCAM, ICAM);
- determination of specific micro-RNAs related to plaque vulnerability;
- echocardiography (+ speckle tracking) for assessment of left ventricular function and size;
- full characterization of PD (dental plaque/tartar, gingival retraction, gingival bleeding etc.);
- microbiological determination of oral bacteria from the periodontal pockets;
- noninvasive imaging by coronary CT angiography for the entire coronary tree, and characterization of vulnerability markers and atherosclerosis severity using surrogate imaging biomarkers such as calcium score, necrotic core, plaque burden, low density atheroma, positive remodeling, epicardial fat volume;
- invasive imaging performed during invasive revascularization procedures, using intracoronary imaging techniques (OCT, IVUS) and quantification of invasive imaging biomarkers in culprit and non-culprit lesions such as macrophage content, thickness of fibrous cap and necrotic core;
- atherectomy of coronary culprit atheromatous plaques (in the atherectomy sub-study), performed during the revascularization procedure when indicated, in selected cases, followed by histological examination of the samples collected in order to identify specific antigens related to oral microbiota in the atheromatous tissue of coronary vulnerable plaques.

Follow-up will be performed at 1, 3, 6, 12, 15, 18, and 24 months after randomization, including assessment of clinical data, echocardiography, and registration of major adverse cardiovascular events and other adverse events.

In addition, complex imaging assessment using CT angiography will be performed at 2 years to assess atherosclerosis progression.

The study procedures are summarized in Table 2.

Statistical analysis will be performed in the Laboratory of Medical Statistics of the Center of Advanced Research in Multimodality Cardiac Imaging of the Cardio Med Medical Center, Tîrgu Mureş, Romania.

STUDY ENDPOINTS/OUTCOME MEASURES

The **primary endpoint** of the study will be represented by the rate of major adverse cardiovascular events (MACE) in PD vs. non-PD patients and in correlation with: (1) the level of systemic inflammation triggered by PD and/or by ACS at baseline; (2) the vulnerability degree of atheromatous plaques in the coronary tree (culprit and non-culprit lesions); (3) the presence and burden of oral bacteria in atheromatous plaques.

MACE will be defined as cardiac death, need for target vessel revascularization (with target vessel defined as the vessel with a vulnerable plaque), or re-infarction.

Secondary endpoints will be represented by: (1) the rate of progression of vulnerability degree of non-culprit coronary plaques; (2) the rate of progression of atheromatous burden and calcium scoring of the coronary tree; and (3) the occurrence of left ventricular remodeling and post-infarction heart failure.

ETHICS

All study procedures are in line with the principles of the Declaration of Helsinki. All patients will sign an informed consent prior to be enrolled in the study. The study received Ethics approval from both institutional boards (approval no. 25/28.12.2017 from the Ethics Committee of the Cardio Med Medical Center, and approval no. 351/13.12.2017 2017 from the University of Medicine and Pharmacy of Tîrgu Mureş).

CONCLUSIONS

The aim of this study is not only to determine the correlation between PD and coronary atherosclerosis but also to analyze the role of PD in triggering an ACS, having as a working hypothesis that both entities have chronic inflammation as a common substrate. ATHERODENT will be the first study to assess the vulnerability degree of atheromatous plaques and of the entire coronary tree in patients with ischemic heart disease and concomitant PD, using a complex imaging platform that will integrate multiple imaging-derived biomarkers in a multimodality approach (CT, IVUS, OCT).

The ultimate goal of the ATHERODENT study is to establish the role of PD not only in triggering an acute coro-

nary event but also in the evolution of coronary atherosclerosis and ventricular remodeling after an ACS.

The findings of the ATHERODENT study can be useful in clinical practice for the assessment of the severity of coronary artery disease and for a better stratification of the cardiovascular risk in patients with coronary atherosclerosis and concomitant PD.

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CONFLICT OF INTEREST

Nothing to declare.

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