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“Cross-sectional and Prospective Associations between Subclinical Atherosclerosis and Periodontal/Peri-implant Inflammation.”

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Introduction

Periodontitis is amongst the most common inflammatory diseases of mankind. It is a chronic inflammatory response of the gingival tissues to a dysbiotic dental biofilm which relentlessly produces soft and hard tissues loss around teeth (Janakiram 2020). The most recent evidence confirm that periodontitis affects 45%-50% of the population (Kassebaum 2014, Kassebaum 2017). As a disease characterised by slow and painless progression, it often goes undetected for several years and is one of the major causes of tooth loss (Janakiram 2020).

Several authors (Loos 2005, D'Aiuto 2006, Lalla 2011, Genco 2013) referred a positive correlation and a direct relationship between periodontitis and systemic diseases over the years: cardiovascular diseases (CVD), hypertension, dyslipidaemia and diabetes mellitus. Shimazaki et al. (Shimazaki 2007) and D'Aiuto et al. (D'Aiuto 2008) reported, for the first time, a correlation between metabolic syndrome (MetS) and periodontal disease in two cross-sectional studies. Several authors (Nibali 2013, Tu 2013, Lamster 2017) highlighted how the prevalence of periodontal disease in patients affected by MetS was almost double, compared to those without Mets (OR=1.7-2.1).

Based on the latest joint workshop of the European Federation of Periodontology (EFP) and the World Heart Federation (WHF) (Sanz 2020), a significant association between periodontitis and CVD has been acknowledged. Consistent evidence suggests that periodontitis is linked not only to a local inflammatory response but also to a systemic host-immune response and increased bacterial burden, which could impact on the progression of atherogenesis and explain the increased risk of vascular complications found in patients with periodontitis (Khader 2004, Dietrich 2013, Lockhart 2012, Orlandi 2014, Herrera 2020, D'Aiuto 2013, Orlandi 2020).

Dental implants are often used as devices to replace missing teeth and their use in the population is steadily increasing (Elani 2018), with almost 2.800.000 dental implants placed per year in Europe.

Mucosal tissues surrounding dental implants however are not free from inflammatory complications, known as peri-implant diseases and further classified in mucositis (Fig. 1) and peri-implantitis (Fig. 2, Fig. 3) (Schwarz 2018, Ferreira 2018).

Fig.1. An implant affected by mucositis

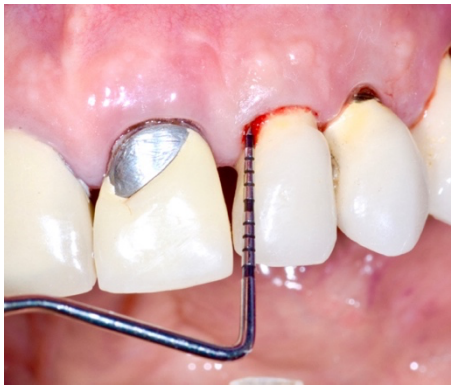


Fig. 2: An implant affected by peri-implantitis

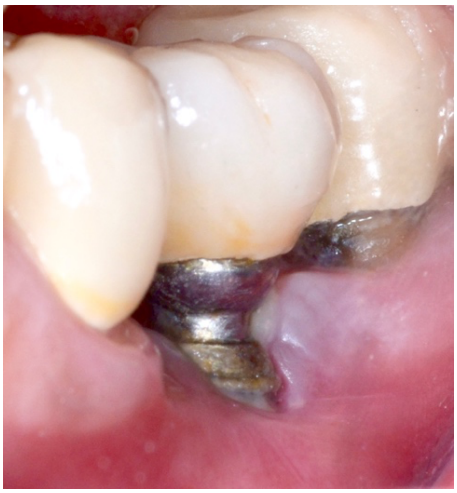
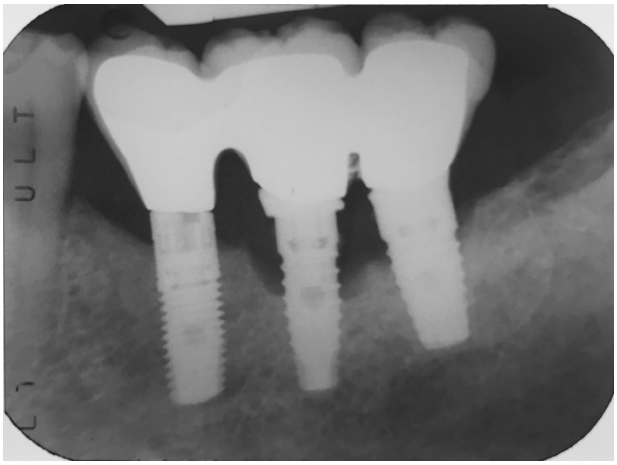


Fig. 3: Radiographic aspect of peri-implantitis



Their prevalence is still controversial and depends mostly on case definitions adopted: Derks & Tomasi reported, in a systematic review of epidemiological studies, that 43% of dental implants are affected by mucositis and 22% by peri-implantitis. On the contrary, a recent systematic review (Rakic 2018) downgraded peri-implantitis rate of 18.5% at the patient-level and 12.5% at the implant-level. According to Schwarz et al. (Schwarz 2018), there is conclusive evidence only for history of periodontitis, poor plaque control and lack of regular maintenance therapy as confirmed risk factors for peri-implantitis, as supported by several longitudinal and cross-sectional studies (Ferreira 2018, Smith 2017, Renvert 2015). Current evidence on the relationship between systemic conditions and peri-implant diseases is limited to sporadic cross-sectional and epidemiological studies and is still controversial. Monje et al. (Monje 2017) found out that diabetes patients no-smokers showed a 3.39 higher risk for peri-implantitis compared to normoglycaemia subjects, while several other studies (Marrone 2013, Costa 2012, Renvert 2014) failed to identify diabetes as a risk factor for peri-implantitis.

Renvert et al. (Renvert 2014) concluded that history of CVD had a high likelihood of comorbidity with peri-implantitis, expressing an OR of 8.7, while Koldsland et al. (Koldsland 2011) found no association between CVD and peri-implant diseases. Very limited evidence is available for the relationship between obesity and peri-implant diseases (de Oliveira PGFP 2020, Alkhudhairy 2018, Vohra 2018), reporting a general increase in peri-implant inflammatory parameters (PPD, BOP, PI, MBL) for obese patients compared to non-obese subjects. Based on the paucity of scientific data currently available on the possible role of systemic conditions as risk factors for peri-implant diseases (Turri 2016, Papi 2018), we decided to focus our attention on investigating the possible role of Metabolic syndrome (MetS) as a risk indicator for peri-implantitis.

In two pivotal studies (Di Murro 2019, Papi 2019), our Research Group highlighted for the first time, the greater prevalence of peri-implant diseases in patients affected by Metabolic Syndrome. Based on our findings, in MetS subjects, peri-implantitis was detected in 36.9% (n = 31) of implants, and mucositis in 60.7% (n = 51), with an OR of 10.01(P = 0.005) for mucositis and OR 15.26 (P = 0.001)

for peri-implantitis, compared with subjects without MetS, where 26.3% of implants showed peri-implantitis and 55.5% mucositis. In this cross-sectional study, we enrolled 183 patients and obtained access to their complete medical records, including their vascular and hypertensive state.

Hypertension is the most prevalent among CVDs, affecting approximately 45% of the worldwide population (Williams ESC/ESH 2018, Mancia 2013), it is the main cause of mortality and/or disability and a major public health concern (GBD 2016). Hypertension management is a cornerstone step in the prevention of cardiovascular (CV) events, such as myocardial infarction and stroke (Luo 2020). It is rather common to observe raised blood pressure values (≥ 130/85 mmHg) clustered with all other classical CVD risk factors (dyslipidaemia, obesity and insulin resistance). Indeed, raised blood pressure is one of the diagnostic criteria of Metabolic syndrome (MetS) affecting almost 40% of the world-wide population, with the highest prevalence in individuals over 50 years of age (Beltran-Sanchez 2013, Samson 2014).

Among other surrogate CVD markers, the ultrasound assessment of the carotid intima-media thickness (c-IMT) has been proposed as a non-invasive research and diagnostic tool to evaluate structural arterial atherosclerosis and is a consistent predictor of future CV events (Polak 2016, Baldassarre 2012). Further, c-IMT has been linked to other cardiovascular risk factors, such as diabetes, hypercholesterolemia and hypertension (Galaska 2020, Yeboah 2008). A c-IMT value greater than 0.9 mm or presence of a Stenotic carotid plaque have shown a strong predictive value for future CV events, independently of other traditional risk factors (Williams ESC/ESH 2018).

Consistent evidence (Orlandi 2014, Orlandi 2020) has been proposed to highlight the role of periodontitis as a risk indicator for subclinical atherosclerosis, suggesting a beneficial effect of periodontal treatment in improving endothelial function. Hence, higher values of c-IMT have been found in periodontal patients, together with other elevated systemic inflammatory parameters, such as serum C-reactive protein (CRP), salivary interleukin (IL)-6 and IL-lβ (Aguileria 2020, Pietropaoli 2020).

Little evidence, however, is available on the potential impact of peri-implant diseases on systemic markers of health or disease. The aim of this dissertation was to investigate, either cross-sectionally and prospectively, the association between subclinical atherosclerosis, evaluated using the surrogate marker carotid intima media thickness (IMT), and periodontal and peri-implant diseases in a sample of patients suffering from primary hypertension.

Material and Methods

Study population

From April 2018 to September 2018, all referrals to the Tertiary Centre of Secondary Hypertension Unit, Policlinico Umberto I, Sapienza University of Rome, for screening, diagnosis, and treatment of primary and/or secondary HT were consecutively evaluated and included in the study by Papi et al. (Papi 2019) if they had ≥ 18 years of age, presenting with a diagnosis of hypertension and the presence of at least one osseointegrated implant in function for >5 years.

The diagnostic criteria of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH) (Williams ESC/ESH 2018) were adopted: Arterial hypertension was defined as systolic BP (SBP) values ≥130 mmHg and/or diastolic BP (DBP) values ≥80 mmHg recorded over the 24h with Ambulatory Blood Pressure Monitoring (ABPM). Secondary forms of hypertension, such as primary aldosteronism, renovascular diseases, Cushing's syndrome, and pheochromocytoma were considered as exclusion criteria.

Each participant signed the informed consent form and the study received ethical approval by the institution review board of "Sapienza" University of Rome (Ref. 4948/2018). The study results were reported according to the STROBE statement (www.strobe-statement.org).

After periodontal examination, patients were instructed to seek treatment of periodontal and peri-implant conditions diagnosed at their referring dental professionals. Only oral hygiene instructions were provided, and no further treatment was implemented.

From January to February 2020 all patients included in the original cross-sectional study by Papi et al. (Papi 2019) were recalled at the Tertiary Centre of Secondary Hypertension Unit to evaluate their medical condition. Patients were asked if they had received periodontal therapy for periodontitis and peri-implant diseases since the baseline examination. Only patients who did not receive periodontal treatment from their dental practitioners were enrolled in the prospective analysis.

Medical Examination

Anthropometric measurements and venous blood samples were obtained from all patients in the early morning after an overnight fast. An experienced physician blinded with respect to periodontal and peri-implant conditions performed the anthropometric measurements and the sonographic assessment. Body Mass Index (BMI) was recorded for each patient (Kg/m^2) and waist circumference was measured placing the measuring tape horizontally around the patient's abdomen and aligning the bottom edge of the tape with the belly bottom. We used a measuring tape with a spring handle in order to control the pressure exerted on the patient's abdomen. Data about smoking habit, as well as current medications (number and type), past medical history, was collected by trained staff.

Vascular assessments

A 24-hours ambulatory blood pressure monitoring (ABPM) and ultrasound assessment of carotid arteries were recorded as markers of hypertensive- and metabolic-related vascular damage (Stein 2008).

The 24-hours ABPM was performed using the Spacelabs 90207 (SpaceLabs®, Washington, USA). For each registration, the blood pressure (BP) values were obtained every 15 minutes during the day and every 30 minutes during the night time period. The parameters collected included: mean 24-hours systolic and diastolic BP and its standard deviation (SD), mean daily and night-time systolic and diastolic BD and their SD, the dipping values.

An ultrasound scan was used to image the common carotid artery, the carotid bulb, and the near and far wall segments of the internal carotid artery bilaterally. Images were obtained in longitudinal

sections with a single lateral angle of insonation, optimizing the image for the far wall. According to the consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force, endorsed by the Society for Vascular Medicine (Stein 2008) IMT was defined as the distance between the ultrasound interfaces of the lumen-intima and media-adventitia. Six manual measurements were performed, with automatic border detection, at equal distances along 1 cm on the far wall of the common carotid. Carotid plaque was defined as the presence of focal wall thickening that is at least 50% greater than that of the surrounding vessel wall or as a focal region with IMT greater than 1.5 mm that protrudes into the lumen that is distinct from the adjacent boundary.

Biomarkers

Serum concentrations of fasting plasma glucose (FPG), total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides (TG), creatinine and blood uric acid level were measured. Urine samples were collected for each patient over the 24-hours to evaluate the 24-h microalbuminuria and patients were also asked to collect a fasting spot urinary sample on the morning of the delivery of the collected urine samples to detect the microalbuminuria spot.

All patients were screened for MetS according the NCEP ATP III criteria (Expert panel 2001). The diagnosis was made by the evidence of ≥ 3 of the following criteria: (1) WC ≥ 102 cm (M) or ≥ 88 cm (F); (2) Fasting plasma glucose value ≥ 110 mg/dL; (3) serum triglycerides concentration ≥ 150 mg/dL; (4) serum HDL - cholesterol concentration < 40 mg/dL (M) or < 50 mg/dL (F) and (5) BP ≥ 130/85 mmHg, obtained by 24-hours ABPM.

Periodontal examination

All patients with at least one dental implant with >5 years of functional loading were referred at the Oral Surgery Unit, Policlinico Umberto I, “Sapienza” University of Rome to evaluate periodontal and peri-implant status.

Patients' data collected included: sex, age, referred medical systemic condition and periodontal status (presence or absence of periodontitis).

A Periodontal Screening and Recording (PSR) index (Landry RG 2002) was collected in each of the sextants by using a periodontal probe with a light force (approximately 0.15 N).

A patient was defined as a "periodontitis case" in accordance with the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions (Tonetti 2018) if:

- Interdental clinical attachment loss (CAL) ≥2 mm was detectable at ≥2 non-adjacent teeth, or
- Buccal or oral CAL ≥3 mm with pocketing >3 mm was detectable at ≥2 teeth and the observed CAL cannot be ascribed to non-periodontal causes such as: 1) gingival recession of traumatic origin; 2) dental caries extending in the cervical area of the tooth; 3) the presence of CAL on the distal aspect of a second molar and associated with malposition or extraction of a third molar, 4) an endodontic lesion draining through the marginal periodontium; and 5) the occurrence of a vertical root fracture.

To achieve intra-examiner reliability, the examiner was calibrated to show an agreement of 90% within 1 mm by duplicate measurements of probing depths on randomly selected teeth (10) and implants (10).

For each implant, the following clinical measurements were recorded:

- Probing Pocket Depth (PPD). Measured in millimetres, is the distance from the mucosal margin to the bottom of the probable pocket.
- Plaque Index (PI) recorded with dichotomic values (present/absent)
- Suppuration defined as a pus formation followed by discharge within a natural aperture or fistula and recorded with dichotomic values (present/absent)
- Bleeding on probing recorded with dichotomic values (present/absent)

Furthermore, years of functional loading, implant location (maxilla or mandible) and type of prostheses (single crown or multiple unit) were recorded.

In addition, mesial and distal implant crestal bone levels were measured on standardized (Rinn, York, PA, USA) digital periapical x-rays for each implant obtained by using an imaging plate scanner (PSPIX²®, Acteon Group, Norwich, UK). A calibrated software (SOPRO Imaging, Acteon Group, Norwich, UK) was used to estimate marginal bone level. Two expert investigators who were blinded to other aspects of the study conducted the radiographic assessment. Any disagreement was solved by consensus, and a third investigator was consulted when it was not initially possible to achieve complete agreement (defined as a difference between the measurements made by the two experts of >0.1 mm).

The reference point for the bone level measurement was the implant shoulder. The bone level was digitally evaluated by measuring the distance between the implant shoulder and the first visible bone contact on the implant. The bone level measurements were recorded on the mesial and distal aspect of each implant.

Case definitions for epidemiological or disease surveillance studies of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions were adopted to establish diagnosis of peri-implant diseases (Berglundh 2018).

Peri-implant health was defined as absence of clinical signs of inflammation, bleeding and/or suppuration on gentle probing, without radiographic bone loss.

Peri-implant mucositis was characterized by presence of bleeding and/or suppuration on gentle probing, without radiographic bone loss.

Peri-implantitis was defined as presence of bleeding and/or suppuration on gentle probing, with radiographic bone levels ≥3 mm apical of the most coronal portion of the intra-osseous part of the implant.

Statistical analysis

Data were evaluated using standard statistical analysis software (version 20.0, Statistical Package for the Social Sciences, IBM Corporation, Armonk, NY, USA). A database was created using Excel (Microsoft, Redmond, WA, USA) and corrected for errors/inconsistencies. Descriptive statistics

including mean ± SD values and percentage were calculated for each variable. The Shapiro-Wilk test was used to determine whether or not the continuous data conformed to a normal distribution.

Two outcome CVD variables were identified as the presence of c-IMT > 0.9 mm and of any carotid atherosclerotic plaque. These were chosen as of their predictive value to future increased cardiovascular risk (Williams ESC/ESH 2018).

Measures of exposure included a variety of continuous and categorical variables collected in the oral examination. A cumulative mucosal inflammatory index (the sum of all PSR values per each patient) was calculated as previously described (Masi 2014). Average PPD and bone levels were calculated as patient-level variables. IMT >0.9 or presence of plaque or the combination between these two variables were modelled against the following independent variables: gender (male/female), diagnosis of periodontitis (yes/no), smoking (yes/no), presence of peri-implant diseases (healthy implant/mucositis/peri-implantitis), cumulative PSR, mean marginal bone loss, BMI, waist circumference, CRP, glucose, total cholesterol, HDL, LDL, Triglycerides, creatinine, blood uric acid level, 24-hour systolic blood pressure, 24-hour diastolic blood pressure, microalbuminuria detection in 24h.

Using variables obtained at the baseline evaluation, a binomial logistic regression was performed to investigate the potential association between each parameter and the dependent variables (IMT > 0.9mm or IMT ≤ 0.9mm with the presence of carotid atherosclerotic plaque versus IMT ≤ 0.9mm and absence of atherosclerotic plaques (Model 1); IMT > 0.9mm versus IMT ≤ 0.9mm (Model 2); presence of carotid atherosclerotic plaque versus absence of carotid atherosclerotic plaque (Model 3)). Linearity of the continuous variables with respect to the logit of the dependent variable was assessed via the Box-Tidwell (1962) procedure. The results of binomial were presented as odds ratio (OR) and 95% confidence interval. Correlation analyses between mean c-IMT values and cumulative PSR were further investigated using Spearman's rank-order testing

The comparison between medical parameters collected at baseline and follow-up was assessed by Mann-Whitney U test for continuous variables and by Chi-square test of homogeneity and Fisher's

exact test for categorical variables. Then, a multiple regression (backward stepwise) was performed to ascertain the effects of independent variables on average c-IMT as continuous outcome.

Statistical significance was set at $p \leq 0.05$.

Results

Original database (Papi et al. 2019)

Subject data

A total of 784 consecutive patients was evaluated at the Secondary Hypertension Unit in the study period (April to September 2018): 363 subjects declared they had dental implants placed, however 134 had implants functioning by less than 5 years and were, therefore, excluded from the study.

The remaining 229 patients were referred at the Oral Surgery Unit in order to evaluate peri-implant status: 26 refused to be included in the study, 20 did not attend the scheduled visit and refused a new dental examination. A final sample of 183 patients was analysed by Papi et al. (Papi 2019): 61.2% of females and 38.8% of males (with a mean age of 66.08 ± 10.42 years (age range 42-85). In the medical history, 45.9% of patients had a diagnosis of metabolic syndrome, while the remaining 54.1% did not meet MetS criteria. At the time of evaluation, presence of periodontitis was detected in 62.8% of subjects.

Implant data

Subjects enrolled had 567 dental implants placed, with a mean of 3.09 implants per patient. The mean functional time was 7.61 ± 4.04 years (range: 5-24 years). Out of the 183 patients included, mucositis was diagnosed in 57.9% of cases, peri-implantitis in 31.1% and only 10.9% of implants were classified as healthy. Mean values of MBL were mesially 1.765 ± 1.424 and distally 1.918 ± 1.576 mm (Fig.4, Fig. 5). The mean PPD was 3.71 ± 1.48 mm.

Fig. 4. Full mouth series of intraoral radiographs of a patient affected by periodontitis and peri-implantitis

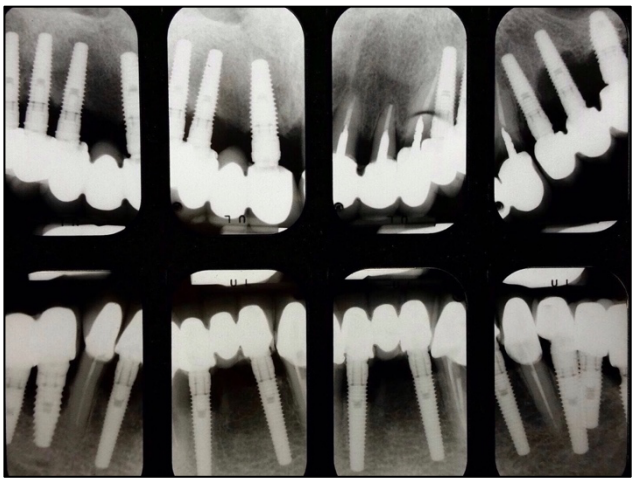
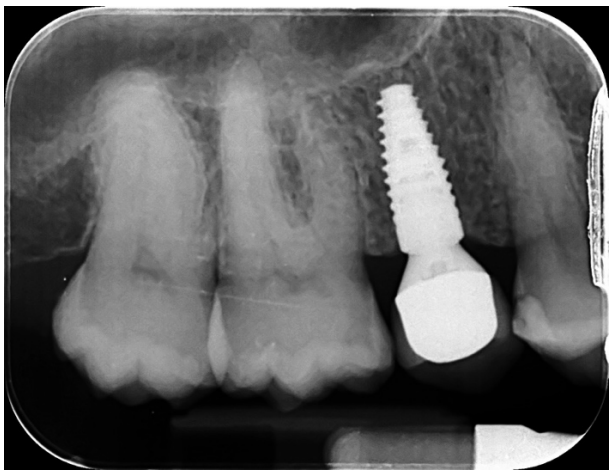


Fig. 5. Periapical x-ray of a healthy implant



Cross-sectional analysis

A total of 151 patients had complete available data on their hypertensive state (c-IMT values, presence of carotid plaque) at the baseline evaluation. In this dataset of patients, the prevalence of periodontitis was 61.6 %, while 57% of participants had mucositis and peri-implantitis was detected in 27.8% of patients. Participants affected by periodontitis had a mean age of 68.78 ± 9.59 years, 61.3% of patients were females, with 43% of smokers, they had a carotid plaque in 71% of cases and mean IMT values were 0.97 ± 0.1 mm. Patients affected by mucositis had a mean age of 68.56 ± 9.50 years, 61.3% of patients were females, 32.6% smokers, presented a carotid plaque in 62.8% of cases and had mean IMT values of 0.94 ± 0.12 mm. Patients affected by peri-implantitis had a mean age of

65.89 ± 10.72 years, 59.5% were females, 33.1%, smokers, presented a carotid plaque in 52.4% of cases and had mean IMT values of 0.93 ± 0.12mm (Table 1, Table 2).

Table 1. Baseline characteristics of participants according to IMT values and presence of carotid plaque (n = 151).

Variable (*mean ± SD)(n,%)*	**IMT > 0.90mm/ plaque (N=103)**	**IMT ≤ 0.90mm/ no plaques (N=48)**	***p value***
Age, years	69.69 ± 9.29	61.16 ± 9.38	**<0.001**
Gender, Male (n; %)	41 (39.8%)	21 (43.8%)	0.723
Smoking, current (n; %)	35 (34.0%)	15 (31.2%)	0.853
CRP, mg/l	2.85 ± 1.40	1.73 ± 1.50	**<0.001**
BMI, kg/m^2	25.78 ± 3.16	27.31 ± 3.79	**0.023**
Waist Circumference, cm	97.09 ± 9.45	99.84 ± 13.24	0.084
Glucose, mg/dL	93.36 ± 18.76	91.81 ± 10.55	0.871
Total Cholesterol, mg/dL	190.50 ± 43.00	199.19 ± 34.05	0.473
Triglycerides, mg/dL	123.02 ± 68.17	148.54 ± 68.39	**0.009**
HDL, mg/dL	59.68 ± 18.19	58.77 ± 18.12	0.521
LDL, mg/dL	107.58 ± 32.92	109.33 ± 36.04	0.525
Creatinine, mg/dL	0.96 ± 0.27	0.87 ± 0.22	0.263
Uric Acid, mg/dL	5.78 ± 1.21	5.60 ± 1.73	0.95
Microalbuminuria spot, mg/dL	19.32 ± 1.21	5.56 ± 1.21	**<0.001**
24 h Microalbuminuria, mg/dL	24.79 ± 50.34	8.9 ± 4.40	**<0.001**
24 h Heart rate, bpm	70.03 ± 5.91	74.49 ± 8.44	**0.008**
24 h Systolic blood pressure, mmHg	128.76 ± 12.53	126.49 ± 10.57	**0.029**
24 h Diastolic blood pressure, mmHg	73.10 ± 8,88	79.21 ± 6.69	**<0.001**
Cumulative PSR	14.88 ± 4.14	11.35 ± 4.51	**<0.001**
Periodontitis, (n; %)	78(75.7%)	15(31.2%)	**<0.001**
Peri-implant diseases, (n; %)			**0.008**
Healthy implants	10(9.7%)	13(27.1%)	
Mucositis	66(64.1%)	20(41.7%)	
Peri-implantitis	27 (26.2%)	15(31.2%)	

Table 2. Baseline characteristics of participants according to periodontal and peri-implant status (n = 151).

Variables (*mean ± SD) (n; %)*	**No Periodontitis N=58**	**Periodontitis N=93**	**Healthy implant N=23**	**Mucositis N=86**	**Peri-implantitis N=42**
Age, years	64.37 ± 10.35	68.78 ± 9.59	64.00 ± 10.48	68.56 ± 9.50	65.89 ± 10.72
Gender, Males	26 (44.8%)	36 (38.7%)	15 (65.2%)	30 (34.9%)	17 (40.5%)
Smoking, current	10 (17.2.0%)	40 (43.0%)	6 (26.1%)	28 (32.6%)	16 (33.1%)
Presence of plaque	20(34.5%)	66(71.0%)	10(43.5%)	54(62.8%)	22(52.4%)
C_IMT, mm	0.84 ± 0.15	0.97 ± 0.10	0.83 ± 0.18	0.94 ± 0.12	0.93 ± 0.12
CRP, mg/l	1.92 ± 1.51	2.87 ± 1.40	1.95 ± 1.35	2.65 ± 1.55	2.54 ± 1.45
BMI, kg/m^2	26.68 ± 3.69	25.98 ± 3.24	27.67 ± 3.61	25.99 ± 3.54	25.91 ± 2.87
Waist Circumference, cm	98.35 ± 12.43	97.66 ± 9.67	102.35 ± 12.96	96.28 ± 9.39	98.74 ± 11.51
Glucose, mg/dL	97.65 ± 21.34	89.98 ± 12.29	92.26 ± 9.80	96.43 ± 19.70	85.74± 9.18
Total Cholesterol, mg/dL	200.35 ± 35.46	188.69 ± 43.01	207.65 ± 38.01	192.43 ± 42.82	185.82 ± 35.55
Triglycerides, mg/dL	145.82 ± 70.89	121.50 ± 66.55	117.91 ± 38.16	127.19 ± 63.91	146.11 ± 89.88
HDL, mg/dL	59.68 ± 18.19	58.77 ± 18.12	61.70 ± 19.44	58.83 ± 17.86	59.24 ± 18.19
LDL, mg/dL	116.65 ± 33.76	102.86 ± 32.87	121.17 ± 31.97	109.04 ± 33.95	98.21 ± 32.22
Creatinine, mg/dL	0.90 ± 0.29	0.95 ± 0.24	0.88 ± 0.20	0.94 ± 0.31	0.93 ± 0.16
Uric Acid, mg/dL	5.62 ± 1.33	5.79 ± 1.43	5.27 ± 1.05	5.65 ± 1.24	6.16 ± 1.73
Microalbuminuria spot, mg/dL	14.98 ± 36.32	15.25 ± 35.18	2.73 ± 4.24	23.66 ± 44.04	4.52 ± 15.02
24 h Microalbuminuria, mg/dL	20.58 ± 46.42	19.62 ± 45.04	3.61 ± 3.07	30.84 ± 55.91	6.77 ± 22.11
24 h Heart rate, bpm	72.19 ± 7.73	70.89 ± 6.60	70.65 ± 7.31	70.86 ± 6.81	72.92 ± 7.36
24 h SBP, mmHg	128.76 ± 12.53	126.49 ± 10.57	126.22 ± 12.12	128.01 ± 12.02	129.13 ± 11.98
24 h DBP, mmHg	77.29 ± 9.61	73.51 ± 7.84	78.17 ± 8.61	72.63 ± 7.84	77.95 ± 9.24
Cumulative PSR	9.39 ± 2.70	16.52 ± 3.06	10.30 ± 3.91	14.30 ± 4.37	14.89 ± 4.34

A statistically significant higher number of patients treated with beta-blocker, calcium channel blocker Diuretic or anticoagulant/antiplatelet drugs presented IMT values > 0.90mm or presence of carotid atherosclerotic plaque. The characteristics of medications taken by participants were summarized in Table 3 and Table 4.

Table 3. Medications use according to IMT values and presence of plaque (n = 151)

	IMT > 0.90mm/ plaque (N=112)	IMT ≤ 0.90mm/ no plaques (N=39)	*p value*
Beta-blocker	38 (36.9%)	5 (10.4%)	0.001
Calcium channel blockers	56 (54.4%)	15 (31.2%)	0.009
Ace inhibitors	15 (14.6%)	4 (8.3%)	0.430
Diuretic	14 (13.6%)	0 (0%)	0.005
Anticoagulant/ Antiplatelet	75 (86.2%)	11 (23.4%)	<0.001
Lipid-lowering	39 (37.9%)	21 (43.8%)	0.592

Table 4. Types of medications taken by participants according to periodontal and peri-implant status.

	Periodontitis			Peri-implant diseases			
	Yes	**No**	***p value***	**Healthy**	**Mucositis**	**Peri-implantitis**	***p value***
Beta-blocker	27 (29.0%)	16 (27.6%)	1.000	4 (17.4%)	25 (29.1%)	14 (33.3%)	0.389
Calcium channel blockers	46 (49.5%)	25 (43.1%)	0.504	10 (43.5%)	47 (54.7%)	14 (33.3%)	0.071
Ace inhibitors	13 (14.0%)	6 (10.3%)	0.618	6 (26.1%)	8 (9.3%)	5 (11.9%)	0.97
Diuretic	10 (10.8%)	4 (6.9%)	0.568	0 (0%)	13 (15.1%)	1 (2.4%)	0.16
Anticoagulant/ Antiplatelet	60 (76.9%)	26 (46.4%)	**<0.001**	11 (57.9%)	50 (65.8%)	25 (64.1%)	0.814
Lipid-lowering	36 (38.7%)	24 (41.4%)	0.864	6 (26.1%)	31 (36.0%)	23 (54.8%)	0.44

Patients with IMT values greater than 0.90mm or with presence of carotid atherosclerotic plaque showed higher level of CRP (p<0.001), Microalbuminuria spot (p<0.001), 24 h Microalbuminuria (p<0.001), 24 h Systolic blood pressure (p=0.029) and, cumulative PSR (p<0.001). Furthermore, this group presented an increased number of patients affected by periodontitis (p<0.001) and peri-implant diseases (p=0.008) (Table 1).

In Model 1, statistically significant associations between IMT > 0.90mm or IMT ≤ 0.90mm with presence of carotid atherosclerotic plaque and Cumulative PSR (OR=1.25, 95% CI:1.12-1.41),

presence of periodontitis (OR=6.71, 95% CI: 2.68-16.76) and presence of mucositis (OR=3.339, 95% CI: 1.13-9.85) were found. The same results could be obtained by applying Model 2, in which the association of IMT > 0.90mm with the same independent variables was evaluated. In Model 3, statistically significant associations between presence of carotid atherosclerotic plaque and cumulative PSR values (OR=1.19, 95% CI: 1.07-1.32) and presence of periodontitis (OR=3.43, 95% CI: 1.47-8.04) were found out, while no association was found for mucositis (p=0.709) or peri-implantitis (p=0.701) (Table 5).

Table 5. Odds ratios (OR) and 95% confidence intervals (CI) of IMT>0.9 or presence of plaque or their combination according to the periodontal and peri-implant status.

	Model 1	Model 2	Model 3
Cumulative PSR	**1.25 (1.12-1.41)*****	**1.32 (1.18-1.47)** ***	**1.19 (1.07-1.32) ****
Presence of periodontitis	**6.71 (2.68-16.76)*****	**8.97 (3.81-21.14)** ***	**3.43 (1.47-8.04) ****
Mucositis	**3.34 (1.13-9.85)***	**3.05 (1.08-8.64)** *	1.24 (0.41-3.75)
Peri-implantitis	1.85 (0.58-5.95)	2.90 (0.94-8.96)	0.79 (0.24-2.63)

Model 1: IMT > 0.90mm or IMT ≤ 0.90mm with the presence of carotid atherosclerotic plaque OR IMT ≤ 0.90mm and absence of atherosclerotic plaques; **Model 2:** IMT > 0.90mm OR IMT ≤ 0.90mm; **Model 3:** presence of carotid atherosclerotic plaque OR absence of carotid atherosclerotic plaque. All models included adjustment for age, gender, smoking, 24 h Systolic blood pressure and BMI. The reference category for Mucositis and Peri-implantitis is: Healthy implants
Statistically significant: * p < 0.05; ** p < 0.01; *** p < 0.001.

The linear regression model confirmed a positive significant association with increased IMT and cumulative PSR values (β=0.011, SE 0.002, p<0.001), presence of periodontitis (β=0.114, SE 0.020, p<0.001) and presence of peri-implant diseases (β=0.011, SE 0.002, p<0.001) (Table 6).

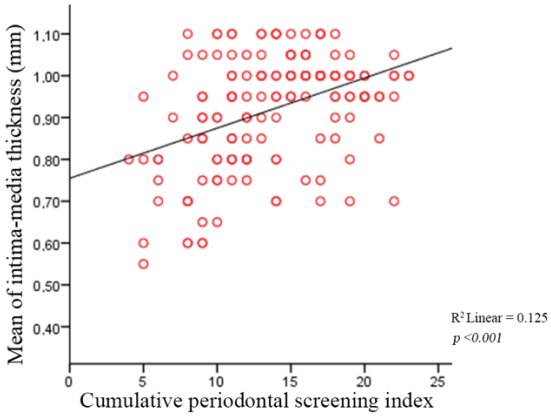
Table 6. Multiple backward stepwise linear regression models (with p = 0.10 to enter and p = 0.05 to leave) of c-IMT according to periodontal and peri-implant status.

	β Coefficient	95.0% CI	*p value*
Cumulative PSR	0.011	0.006-0.015	<0.001
Presence of periodontitis	0.114	0.036-0.157	<0.001
Peri-implant diseases	0.035	0.004-0.066	0.028

Model: reduced model that best explains the data.

Furthermore, a Spearman's rank-order correlation was run to assess the relationship between IMT and cumulative PSR values. There was a statistically significant, strong positive correlation between increase values of IMT and PSR, $r_s = .354$; $p < 0.001$ (Fig 6).

Fig. 6. Scatter plot of c-IMT (mm) values by cumulative PSR



Prospective analysis

All patients composing the original sample evaluated at the baseline visit with complete medical record (N= 151 patients) were recalled at the Secondary Hypertension Unit for a follow-up visit (January-February 2020). Fifty-seven patients were currently subjected to periodontal treatment or enrolled in a maintenance program and were, therefore, excluded from the study. Twenty-tree patients did not attend the scheduled visit and refused a new medical examination. Therefore, a final sample of 71 patients attended the follow-up visit and was included in the study: 44 females (61.97%) and 27 males (38.03%), with a mean age of 53.35 ± 7.92 years (range: 31 – 76 years). Descriptive statistics of variables collected is reported in Table 7.

Table 7. Follow-up characteristics of participants (N= 71).

Variable	**(***mean ± SD)/ (n,%)*
Age, years	53.35 ± 7.92
Gender, Male	27 (38.03%)
Gender, Female	44 (61.97%)
Smoking, current (n; %)	27 (38.0%)
CRP, mcg/l	3376.19 ± 6511.52
BMI, kg/m^2	26.48 ± 3.78
Waist Circumference, cm	93.36 ± 11.94
Glucose, mg/dL	98.90 ± 23.25
Total Cholesterol, mg/dL	179.57 ± 32.96
Triglycerides, mg/dL	122.43 ± 82.42
HDL, mg/dL	51.76 ± 9.78
LDL, mg/dL	103.10 ± 31.47
IMT, mm	0.76 ±0.13
Creatinine, mg/dL	0.93 ± 0.25
Uric Acid, mg/dL	5.26 ± 1.48
24 h Microalbuminuria, mg/dL	14.76 ± 28.94
24 h Heart rate, bpm	69.97 ± 14.94
24 h Systolic blood pressure, mmHg	138.99 ± 21.69
24 h Diastolic blood pressure, mmHg	83.71 ± 12.87
Baseline cumulative PSR	11.83±4.84

Among parameters investigated, only IMT values were statistically significant higher (p= 0.015) at follow-up when compared with baseline data (mean IMT values of 0.76 ±0.13 mm, 0.88 ± 0.19 mm, respectively) (Table 8).

Table 8. Comparison between baseline and follow-up variables among patients successfully recalled

Variable (*mean ± SD)(n,%)*	**Baseline**	**Follow-up**	***p value***
CRP, mcg/l	5285.71± 9296.19	3376.19 ± 6511.52	.107
BMI, kg/m^2	26.60 ± 3.42	26.48 ± 3.78	.893
Waist Circumference, cm	99.81 ± 11.96	93.36 ± 11.94	0.158
Glucose, mg/dL	94.43 ± 15.30	98.90 ± 23.25	0.471
Total Cholesterol, mg/dL	188.00 ± 40.39	179.57 ± 32.96	0.505
Triglycerides, mg/dL	145.76 ± 70.88	122.43 ± 82.42	0.066
HDL, mg/dL	52.14 ± 13.32	51.76 ± 9.78	0.916
LDL, mg/dL	110.52 ± 39.02	103.10 ± 31.47	0.473
IMT, mm	0.76 ±0.13	0.88 ± 0.19	**0.015**
Presence of plaque	27 (38.02%)	34 (47.88%)	0.544
Creatinine, mg/dL	0.94 ± 0.27	0.93 ± 0.25	0.900
Uric Acid, mg/dL	6.01 ± 1.70	5.26 ± 1.48	0.131
24 h Microalbuminuria, mg/dL	9.01 ± 19.70	14.76 ± 28.94	0.464
24 h Heart rate, bpm	70.81 ± 7.63	69.97 ± 14.94	.385
24 h Systolic blood pressure, mmHg	127.90 ± 14.37	138.99 ± 21.69	0.054
24 h Diastolic blood pressure, mmHg	78.29 ± 10.17	83.71 ± 12.87	.106

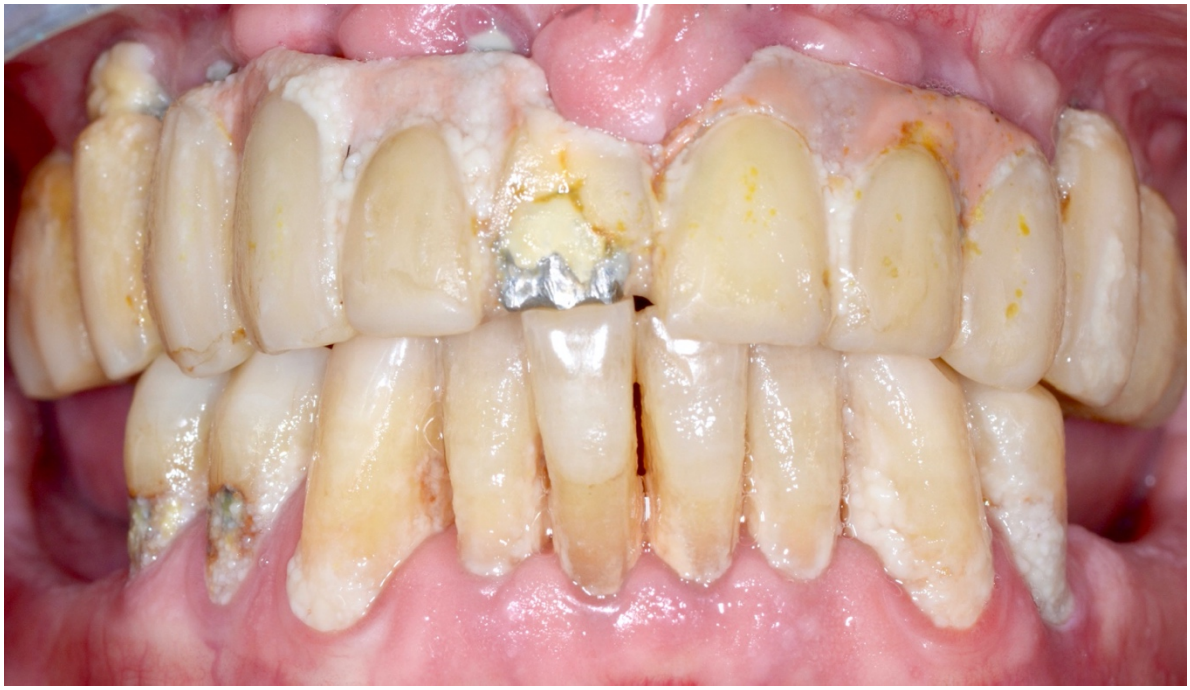
The linear regression model revealed a positive significant association with increased IMT and cumulative PSR values, CRP, Triglycerides and Creatinine, with an $R^2 = 0.863$.

Discussion and Conclusions

The aims of this dissertation were to analyse either cross-sectionally and prospectively the relationship between periodontal and peri-implant inflammation and subclinical atherosclerosis. As previously outlined, current evidence on the association between peri-implant diseases and systemic conditions is still lacking. Hence, this is the first project to investigate the role of peri-implant inflammation as a predictor for subclinical atherosclerosis, evaluated using c-IMT as a surrogate vascular imaging outcome.

In the cross-sectional analysis, three different statistical models were used to determine which independent predictors were associated with increased values of IMT, using plaque presence or IMT > 0.9 mm or the combination of these factors as thresholds of subclinical atherosclerosis. In all cases, periodontitis (OR ranging from 3.43 to 8.97) and cumulative PSR values (OR range: 1.19-1.32) were statistically significant, while peri-implant mucositis was found to be significant in Model 1 (OR= 3.34) and Model 2 (OR= 3.05) and peri-implantitis was not statistically significant in any model. In the linear regression model, a positive significant association with increased IMT was found out for cumulative PSR values (β=0.011, SE 0.002, $p<0.001$), presence of periodontitis (β=0.114, SE 0.020, $p<0.001$) and presence of peri-implant diseases (when considering mucositis and peri-implantitis aggregated) (β=0.011, SE 0.002, $p<0.001$). In this scenario, gingival inflammation was strongly related with systemic inflammation, as outlined by the Spearman's rank-order correlation ($r_s = .354$; $p < 0.001$) (Fig. 7).

Fig. 7. Clinical aspect of a patient affected by either periodontitis and peri-implantitis



Interestingly, patients included in the study were all enrolled at the Secondary Hypertension unit and they were all diagnosed with primary hypertension, therefore excluding secondary forms of hypertension, such as primary aldosteronism, renovascular diseases, Cushing's syndrome, and pheochromocytoma. There were no statistically significant differences in distribution of medications among patients with IMT >0.9 mm/ plaque presence or IMT < 0.9 mm/plaque absence, with the exception of anticoagulants, diuretic and beta-blocker. When considering types of medications taken by participants according to periodontal and peri-implant status, only anticoagulants were statistically more distributed in patients affected by periodontitis.

Over the years, different systematic reviews of observational studies (Orlandi 2014, Zeng 2016) highlighted how patients affected by periodontitis showed higher c-IMT values.

Based on our results, gingival inflammation, expressed as cumulative PSR, was strongly associated with higher c-IMT values. In a recent narrative review, Herrera et al. (Herrera 2020) presented all causes proposed over the years to explain the interrelationship between hypertension and periodontitis. Hence, periodontal disease and atherosclerosis share several pathological mechanisms:

the increased levels of systemic inflammatory mediators (CRP, IL-1, IL-6, TNF-α), dyslipidaemia, upregulation of thrombotic and haemostatic factors. Furthermore, periodontitis might induce systemic bacteraemia, influencing directly the progression of the atheroma plaque, with the latest joint workshop of the EFP/ WHF (Sanz 2020) confirming the available evidence on the presence of periodontal pathogens (Porphyromonas gingivalis, Prevotella Intermedia, Fusobacterium nucleatum, Treponema Denticula) in atheroma lesions. Therefore, the results of our cross-sectional study were consistent with the available literature, however we reported data on the influence of peri-implant inflammation on higher IMT values for the first time, introducing additionally an aggregate measure of gingival inflammation (cumulative PSR). Main limitations were the study design, with the impossibility to draw cause and effect relationship in a cross-sectional study, and the absence of complete periodontal charting.

To overcome limitations imposed by the cross-sectional nature of the study, we designed a prospective cohort study to evaluate longitudinally the influence of the diagnosis of periodontitis and peri-implant diseases on the progression of subclinical atherosclerosis.

All patients included in the cross-sectional analysis were recalled for a follow-up visit to evaluate their medical condition. They were all untreated for periodontitis and peri-implant diseases, since only oral hygiene instructions were provided at the time of the original visit and patients were sent back to their referring dentists. After a mean period of 2 years, patients were recalled, a novel vascular assessment and new biomarkers were obtained. Over the years, different randomized control clinical trials (Kapellas 2014, Tonetti 2007) and longitudinal studies (Kudo 2018, Piconi 2009, Desvarieux 2013) demonstrated benefits of periodontal therapy in reducing IMT values and systemic inflammation and preventing the onset of CV events caused by subclinical chronic atherosclerosis. However, there was no universally accepted definition of periodontitis among these authors:

- Kudo et al.: “Periodontal disease was defined by pocket depth”, 6 sites per tooth, type of periodontal probe not specified

- Kapellas et al.: “periodontitis case status defined as the presence of at least 2 interproximal sites with clinical attachment loss (CAL) ≥ 4 mm, or at least two interproximal sites with probing depth (PD) ≥ 5 mm”, 4 sites per tooth, periodontal probe with 2 mm markings
- Piconi et al.: “Clinical examination was carried out using the periodontal screening and recording (PSR) criteria.”, 4 sites per tooth, Periodontal probe Ty0112
- Desvarieux et al.: “full-mouth periodontal assessment for all teeth present included probing depth (PD) and attachment loss (AL)”, 6 sites per tooth, periodontal probe PCP-15

Furthermore, the collection of medical data was not univocal and complete medical records of patients enrolled were not available. The imprecise measures of periodontitis and hypertension could have influenced the results of their studies and, more importantly, their interpretation. To overcome these limitations, this was the first study to adopt the novel definition of periodontitis (Tonetti 2018) and to present data on peri-implant diseases, showing complete medical records of patients, including the 24-hours ambulatory blood pressure monitoring.

Among parameters investigated, only IMT values were statistically significant higher ($p= 0.015$) at follow-up when compared with baseline data (mean IMT values of 0.76 ±0.13 mm, 0.88 ± 0.19 mm, respectively). Based on our findings, a mean c-IMT progression of 0.12 mm was detected in our cohort of patients after 2 years, therefore representing an average increase of 0.06 mm/year.

A mean c-IMT progression rate of 11.8 μm/year was reported in the The Multi-Ethnic Study of Atherosclerosis (MESA) (Tattersall 2014), while in a meta-analysis of individual-participant-data from 20 prospective studies of the PROG-IMT collaboration (Willeit 2016) the average increase rate was 0.011 mm/year. More recently, the Early Vascular Ageing (EVA)-Tyrol study (Staudt 2020) reported, in a large cohort of young healthy adolescents, a modest increase in c-IMT of 2.78 μm/year. Several factors contribute to fight IMT progression, such as medications lowering blood pressure and lipids: Lind (Lind 2020) reported a significant reduction in IMT over a 10 years period when comparing patients receiving statin and the never-statin group ($p < 0.0001$), Hong et al. (Hong 2019) highlighted in an RCT the effects of antiplatelet drugs on lowering IMT progression, while Huang

(Huang 2016) described how the use of antihypertensive medication was associated with less annual IMT progression. In our study sample, based on periodontal and peri-implant status there were not statistically significant differences at baseline in types of medications taken by participants, with the exception of antiplatelet drugs more diffused in the periodontitis group.

At the follow-up visit, distribution of medications was generally unchanged between participants enrolled, with only some minor and not statistically significant differences ($p>0.05$) when compared with the baseline examination. These findings were confirmed by the hypertensive and lipidic profiles of patients, showing no statistically significant differences between the baseline and the follow-up visits. Based on the results of the prospective analysis, we can cautiously conclude that gingival inflammation is related with the progression of subclinical atherosclerosis. Among a cohort of patients untreated for periodontitis and peri-implant diseases, with no differences for hypertensive and lipidic profiles and medications taken, there was a mean progression rate of 0.06 mm/year during the two years observation period, consistently higher compared to values reported in large epidemiological studies. When taking into account all cofounding variables, the linear regression model revealed a positive significant association with increased IMT and cumulative PSR values, CRP, Triglycerides and Creatinine. Further studies, with larger sample and an interventional design are needed to confirm the findings of this dissertation.

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Appendix

Periodontal Screening and Recording System

SCORE	CRITERIA
0	Pocket depth is < 3.5 mm, no bleeding upon probing, and no calculus
1	Pocket depth is < 3.5 mm, bleeding on probing and no calculus
2	Pocket depth is < 3.5 mm, bleeding on probing and calculus present
3	Pocket is 3.5 - 5.5 mm in depth
4	Pocket is > 5.5 mm in depth
*	Clinical abnormalities, such as furcation involvement, tooth mobility, mucogingival involvement, or 3.5 mm or more of recession in that sextant
X	Edentulous sextant