Dissertation

PREVALENCE OF PERIODONTITIS AND IMPACT OF PERIODONTAL TREATMENT IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASES

Submitted by

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Statuary Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used throughout this thesis and in all related publications I followed the “Standards of Good Scientific Practice at the Medical University of Graz“.

Disclosures

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LIST OF ABBREVIATIONS IN ALPHABETICAL ORDER

AAP	American Academy of Periodontology
ABI	Ankle brachial index
ACVD	Atherosclerotic Cardiovascular Disease
ADA	American Dental Association
BOP	Bleeding on Probing
CAL	Clinical Attachment Level
CG	Control Group
CHX	Chlorhexidine
CPITN	Community Periodontal Index of Treatment Needs
CRP	C-reactive Protein
CVD	Cardiovascular Diseas
DMFT	Decayed Missing Filled Teeth
DMS V	Deutsche Mundgesundheitsstudie V
DANN	Deoxyribo-nucleic-acid
EFP	European Federation of Periodontology
FMD	Full-mouth Disinfection
FWF	Fonds Wissenschaftlicher Förderung
GCF	Gingival Crevicular Fluid
Hs-CRP	high sensitive – C-reactive Protein
IC	Intermittent Claudication
IL-1	Interleukin - 1
IL-6	Interleukin - 6
MMP-8	Matrix Metalloproteinase – 8

NHANES	National Health and Nutrition Examination Survey
OSFMD	One-stage Full-mouth disinfection
PAD	Peripheral arterial Disease
PCR	Polymerase Chain Reaction
PD	Probing depth
PGE2	Prostaglandine E2
PGU	Parodontale Grunduntersuchung
PISA	Periodontal Inflammed Surface Area
PISF	Periimplant Sulcus Fluid
PSR	Periodontal Screening and Recording
PST	Periodontitis Susceptibility Test
PT1	Periodontal Therapy 1
PT2	Periodontal Therapy 2
PTX-3	Pentraxin – 3
RBL	Radiographic Bone Loss
SD	Standard Deviation
SRP	Scaling and Root Planning
TE	Tris(hydroxymethyl)aminomethane Ethylenediaminetetraacetic acid
TIA	Transient ischemic attack
TNF	Tumor necrosis factor
WHO	World Health Organization
ZMF	Centre for medical research

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1. ZUSAMMENFASSUNG

HINTERGRUND

Parodontale Erkrankungen zählen zu den häufigsten weltweit. Die Zusammenhänge und Mechanismen zwischen Parodontitis und systemischen Erkrankungen wurden und werden intensiv beforscht und entwickelten sich zu einem eigenständigen Forschungsfeld: die Parodontale Medizin. Periphere arterielle Verschlusserkrankungen (PAVK)und Parodontitis stehen in enger Beziehung zu einander, sodass eine Reihe von Risikofaktoren für beide Erkrankungen als modulierend bzw. aggravierend einzustufen sind. Ziel dieser Studie, die dieser Dissertation zu Grunde liegt, ist die Erhebung der Prävalenz parodontaler Erkrankungen bei Patientinnen und Patienten mit PAVK und die Untersuchung des Einflusses einer parodontalen Therapie auf klinische, parodontale Parameter.

METHODIK

Zur Prävalenzerhebung parodontaler Erkrankungen konnten Daten von 409 gescreenten Patientinnen und Patienten mit PAVK, Stadium II und III nach Fontaine, und mindestens einem natürlichen Zahn herangezogen werden. Einteilung der Parodontalen Erkrankung wurden anhand der Parodontalen Grunduntersuchung vorgenommen (PGU).

Für die Untersuchung zum Einfluss auf parodontale Parameter wurden 90 Patientinnen und Patienten nach strikten Einschluss- und Ausschlusskriterien randomisiert zu jeweils 30 Patientinnen und Patienten in drei Gruppen. Bei einer Gruppe wurde eine nicht – chirurgische parodontale Therapie durchgeführt unter Einsatz von adjuvanter, systemischer Antibiose (PT1), die zweite Gruppe wurde ebenfalls nicht – chirurgisch parodontal therapiert, jedoch ohne Einsatz von Antibiotika (PT2) und die dritte Gruppe wurde über einen Zeitraum von 3 Monaten nicht behandelt und stellte die Kontrolle dar (CG). Klinische, mikrobiologische und immunologische Parameter wurden zu Beginn, nach 3 Monaten und nach 12 Monaten erhoben.

ERGEBNISSE

Prävalenz: bei 296 männliche und 113 weibliche Probanden zwischen 39 und 89 Jahren (mittleres Alter 63) wurden PGU, DMFT und Anzahl der natürlichen Zähne erhoben. Mittlerer PGU war 3.1, PGU 0 wurde bei 1, PGU bei 10, PGU 2 bei 115, PGU 3 bei 64 und PGU bei 219 Probanden erhoben. Der mittlere DMFT Wert war 22.8 und die mittlere Anzahl der natürlichen Zähne inklusive dritter Molaren und dentalen Implantaten lag bei 16.7.

Einfluss parodontaler Therapie: 319 Probanden aus dem Screening Kollektiv konnten aufgrund dentaler, systemischer oder anderer Ausschlusskriterien nicht berücksichtigt werden. PT1 Probanden erzielten signifikant bessere klinische, mikrobiologische und immunologische Werte als PT2 und CG. PT2 hingegen erzielte ebenfalls bessere klinische Werte als CG. Keine nachteiligen Wirkungen der Therapie konnten beobachtet werden. Bei der CG wurden über den Zeitraum bis 3 Monaten keine signifikanten Veränderungen beobachtet.

ZUSAMMENFASSUNG

Wir konnten zeigen, dass Parodontitis eine hoch – prävalente Erkrankung bei Patientinnen und Patienten mit PAVK, Stadium II und III nach Fontaine ist. Vergleichsstudien zur Allgemeinbevölkerung sind in Österreich mangels Erhebungen nicht möglich. Der Vergleich mit europäischen Nachbarländern zeigt jedoch, dass die Prävalenz deutlich über dem Durschnitt liegt und das DMFT erhöht und Anzahl natürlicher Zähne stark erniedrigt sind.

Die Behandlung unter adjuvanter Antibiose zeigte in den erhobenen Werten eine signifikante Verbesserung und deckt sich zum Teil mit erwartbaren Ergebnissen aus Vergleichsstudien. Mikrobiologische Daten zeigten, dass nur unter Einsatz von systemischer Antibiose, Änderungen von parodontal – pathogener Keime mit Bestimmung via Polymerase Chain Reaction (PCR) Methoden sich zeigen konnten.

Der Einfluss von parodontaler Behandlung bei Patientinnen und Patienten mit PAVK auf vaskulärer Ebene bleibt Gegenstand der Forschung.

2. ABSTRACT

BACKGROUND

Periodontal diseases are among the most common worldwide. The connections and mechanisms between periodontitis and systemic diseases have been and are being intensively researched and have developed into an independent field of research: periodontal medicine. Peripheral arterial occlusive disease (PAD) and periodontitis are closely related to each other, so that a number of risk factors for both diseases can be classified as modulating or aggravating. The aim of this study, on which this dissertation is based, is to investigate the prevalence of periodontal disease in patients with PAD and the influence of periodontal therapy on clinical periodontal parameters.

METHODOLOGY

Data from 409 screened patients with PAVK, stage II and III after Fontaine, and at least one natural tooth were used to determine the prevalence of periodontal disease. Periodontal disease was classified according to the Periodontal Basic Examination (PGU).

For the study on the influence on periodontal parameters, 90 patients were randomized according to strict inclusion and exclusion criteria to 30 patients each in three groups. One group underwent non – surgical periodontal therapy using adjuvant systemic antibiotics (PT1), the second group underwent non-surgical periodontal therapy without antibiotics (PT2) and the third group was left untreated for a period of 3 months and presented the control (CG). Clinical, microbiological and immunological parameters were collected at baseline, after 3 months and after 12 months.

RESULTS

Prevalence: 296 male and 113 female volunteers between 39 and 89 years of age (mean age 63) were tested for PGU, DMFT and number of natural teeth. Mean PGU was 3.1, PGU 0 was found in 1, PGU 1 in 0, PGU 2 in 115, PGU 3 in 64 and PGU 4 in 219 probands. The mean DMFT value was 22.8 and the mean number of natural teeth including third molars and dental implants was 16.7.

Influence of periodontal therapy: 319 subjects from the screening group could not be considered due to dental, systemic or other exclusion criteria. PT1 subjects achieved significantly better clinical, microbiological and immunological scores than PT2 and CG. PT2, on the other hand, also achieved significantly better clinical results than CG. No adverse effects of the therapy were observed. No significant changes were observed in CG over a period of up to 3 months.

SUMMARY

We were able to show that periodontitis is a highly prevalent disease in patients with PAVK, stage II and III after Fontaine. Comparative studies of the general population are not possible in Austria due to a lack of epidemiological studies. However, the comparison with neighbouring European countries shows that the prevalence is well above the average and that the DMFT is increased and the number of natural teeth is highly reduced.

Treatment with adjuvant antibiotics showed a significant improvement in the values obtained and partly coincides with expected results from comparative studies. Microbiological data showed that only with the use of systemic antibiosis, changes of periodontal - pathogenic bacteria with determination via Polymerase Chain Reaction (PCR) methods could be shown.

The influence of periodontal treatment in patients with PAD on the vascular level remains the subject of research.

3. INTRODUCTION

Periodontal disease is an inflammatory disease caused by a bacterial infection of the teeth supporting tissues (Offenbacher, S., 1996). The prevalence of periodontitis in adults is over 46% (Eke, P. I. et al., 2015) and is a major dental public health problem (Eke, P. I. et al., 2012). It is assumed that in ancient times the prevalence of periodontitis was only around 5% (Raitapuro-Murray, Molleson, & Hughes, 2014) and that the potential risk factors like smoking, diabetes and other systemic civilization diseases led this high prevalence, that we are facing in modern days. Not only because of its high prevalence, periodontitis is associated with an array of systemic conditions and diseases like cardiovascular disease, diabetes, psoriasis, rheumatoid arthritis, pregnancy outcomes and respiratory diseases (Falcao & Bullón, 2019). The hypothesis that oral infections have effects that go beyond the oral cavity is not new (Miller, 1891) (O'REILLY & Claffey, 2000) and is supported by evidence which has been gained through a growing number of interventional studies. Some of the most sophisticated and significant publications have been compiled by the European Federation of Periodontology (EFP) and the American Academy of Periodontology (AAP) in a jointly held workshop (Linden, Herzberg, & Working Group 4 of the Joint EFP/AAP Workshop, 2013). In a more recent review systemic disorders and medications that may affect the periodontal tissues have been summarized and many of them are associated with a profound loss of periodontal attachment and alveolar bone (Albandar, Susin, & Hughes, 2018).

In today's understanding periodontal disease is associated with a dysbiosis of the oral microbiome (Kilian et al., 2016). The oral cavity provides various habitats for microbial colonization (Dewhirst et al., 2010) and to date more than 770 prokaryotic taxa have been described in the literature thanks to newly developed sequencing methods which colonize the oral cavity and the human aerodigestive tract (Escapa et al., 2018). So far, the direct effect of dysbiotic oral microbiota on systemic diseases is not fully understood but good oral hygiene is stated to be important to prevent bacterial dissemination to other parts of the body(Han & Wang, 2013).

The EFP launched a manifesto for Periodontal Health for a Better Life in 2012, which can be signed individually online. This campaign intends to contribute to enlargement of the awareness, that periodontal health is one key element of oral and general health and wellbeing (Chapple & Wilson, 2014). The negative consequences for three general health

conditions, gylcaemic control in type-2 diabetes, cardio-vascular diseases and adverse pregnancy outcomes are highlighted in the EFP manifesto having regard to biological plausibility, epidemiological data and results from interventional studies.

Atherosclerotic cardiovascular diseases (ACVD) includes following diseases: fetal and non-fetal coronary heart disease (angina and myocardial infarction), ischemic cerebro-vascular disease (stroke and transient ischemic attack/TIA) and peripheral arterial disease (PAD).

An association between periodontal disease and ACVD has been outlined in reviews (Genco, Offenbacher, & Beck, 2002; Reyes, Herrera, Kozarov, Roldán, & Progulske-Fox, 2013a) (Schenkein & Loos, 2013a) and the most supported theory is based on the metastatic entry of bacteria and their products into the blood stream. These circumstances may lead to an activation of inflammatory mechanisms and a formation, growth and aggravation of atheromateous plaques in the blood vessels. There is moderate evidence that treatment of periodontitis leads to a reduction of inflammatory laboratory markers, like C-reactive protein (CRP)(D'Aiuto, Ready, & Tonetti, 2004) and an improvement of vascular endothelial function(Tonetti, Maurizio S., D'Aiuto, Nibali, Donald, Storry, Parkar, Suvan, Hingorani, Vallance, & Deanfield, 2007a) (Seinost et al., 2005a).

From 2003 and 2005 an interventional pilot study with five clinical centers in the United States of America intended to organize a definitive clinical trial of periodontal treatment in patients with documented cardio – vascular – disease (CVD) for secondary prevention, to design and implement a pilot randomized controlled trial to test the efficacy in recruitment, enrolling patients and data management and to obtain information on feasibility, periodontal and cardiovascular outcomes and adverse events(Couper et al., 2008). A total of 303 of intended 600 participants were randomized. The authors come to the conclusion that it is feasible to conduct a secondary prevention trial of periodontal treatment in patients with coronary heart disease.

This dissertation is part of a joint clinical research project of the division of Angiology and the division of Restorative Dentistry, Periodontology and Prosthodontics, Medical University of Graz, which was funded by the Austrian Science Fund (FWF). The aim of this dissertation is to obtain epidemiological information about the periodontal burden in patients with PAD and in a second stage get insights on the effect of periodontal treatment regarding to periodontal, microbiological and immunological parameters.

a. PERIODONTITIS

i. Definition and Pathomechanism

Periodontitis is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus(Papapanou et al., 2018a). This microbial infection is followed by destruction of soft tissue caused by leukocytes and the generation of cytokines, eicosanoids, and matrix metalloproteinase that cause connective tissue and alveolar bone disintegration(Kornman, Page, & Tonetti, 1997). Neutrophils have a key role in the host response on against invading periodontopathogenic microorganisms(Friedewald et al., 2009). The presence of inflammatory mediator like Interleukin -1β (IL-1 β), which plays a predominant role in defining different disease states, is increased in periodontal diseases (Offenbacher, Steven, Barros, & Beck, 2008). But even if key inflammatory mediators are more present, the host-immune response stays very individually.

Genetic predispositions may lead to an exaggerated reaction of inflammatory response by differences in their expression and many polymorphisms have been determined to be able to increase the risk for periodontal inflammation (Schenkein, 2002). Epigenetic changes also contribute to the regulatory processes that are critical for inflammatory response(Bobetsis et al., 2007). Epigenetic changes may be heritable(Robertson, 2005) or more likely environmental and occur throughout the life of an individual as a response to factors like diet, aging, and toxins (Edwards & Myers, 2007).

Assessment of periodontal inflammation and tissue destruction is based on clinical evaluation of signs and symptoms, including a general medical history. Typical features of inflammatory processes like bleeding and/or suppuration, swelling, redness and sometimes pain can be identified through probing of the pocket depth (PD), checking of bleeding on probing (BOP) and clinical attachment level (CAL).

These techniques certainly have a lack in consistency and reproducibility but are in the same way easy to conduct and therefore very practical.

One of the main shortcomings of the definition of periodontal disease was the absence of a clear definition of periodontal health. In the preamble to the constitution of the World Health Organization, health is defined as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.

Very recently, a practical definition of periodontal health as a state of absence of inflammatory periodontal disease was published. The authors propagate, that there are four levels of periodontal health: 1) pristine periodontal health, defined as a total absence of clinical inflammation and physiological immune surveillance on a periodontium with normal support (no attachment or bone loss). Pristine periodontal health is not likely to be observed clinically; 2) clinical periodontal health, characterized by an absence or minimal levels of clinical inflammation in a periodontium with normal support; 3) periodontal disease stability in a reduced periodontium; 4) periodontal disease remission/control in a reduced periodontium (Lang & Bartold, 2018). Since changes of the biofilm attached supra- and subgingival lead to inflammation limited either only on gingiva or if the virulence increases disintegration of collagen and destruction of periodontal attachment, maintaining a good oral hygiene stays one of the key parameters for periodontal health. Good oral hygiene can be achieved by a good personal - and professional care. For the very first time since periodontal diseases were classified, the authors of this definition outlined that periodontal health can exist before disease outbreak and can be restored to an anatomically reduced periodontium.

ii. **Treatment**

Over 30 years ago a radical change in standard treatment of periodontitis took place. It could be shown, that non – surgical periodontal therapy was as effective as surgical periodontal therapy(Lindhe & Nyman, 1985). Subgingival debridement is today the key element in nonsurgical treatment of periodontal disease and is also used during surgery and regular maintenance (Laleman et al., 2017) .

Any treatment of dental hard tissues surfaces for plaque or calculus removal will result in substance loss. Loss of cementum should be limited to the 3–7 µm layer of bacterial invasion in the cementum tissues underneath the biofilm. The cervical aspects of the cementum layer are thinner than the apical parts and any excessive treatment can minimize or eliminate the cementum layer and initiate resorption by connective tissue or ankylosis.

Roughness of the root surface is a crucial factor in terms of healing and recolonization of bacteria and biofilm development. With non – abrasive powders and air – polishing devices one can achieve roughness values that are comparable to pristine surfaces (Bühler, Schmidli, Weiger, & Walter, 2015). Even the additional use of abrasive polishing paste is questioned as the cleaning efficacy with air – polishing powders seems to be impeccable (Camboni & Donnet, 2016).

iii. **Classification**

Classification systems are the framework of etiology, pathogenesis, diagnostics and therapy of diseases. In 2017 a joint World Workshop of the AAP and the EFP on the Classification of Periodontal and Peri-Implant Diseases was held in Chicago which substitutes the former classification from 1999 (Armitage, 1999). In the new classification system three different forms of periodontitis are described: necrotizing periodontitis, periodontitis as a direct manifestation of systemic diseases and periodontitis. With this new system one should be able to identify clearly if a patient is a periodontitis case, what specific type he or she is and to describe the clinical presentation and elements that could alter the clinical management and prognosis with influence on oral and/or systemic health(Papapanou et al., 2018b).

Periodontitis and systemic diseases have a lot of risk factors in common, like genetics or environmental and can occur simultaneously. The new classification differentiates the manifestations in several subdivisions (Albandar et al., 2018) :

- Systemic diseases and conditions that affect the periodontal attachment apparatus
    - Systemic disorders that have a major impact on the loss of periodontal tissue by influencing periodontal inflammation
        - genetic disorders (Down syndrome, Papillon-Lefèvre syndrome, congenital neutropenia, etc.)
        - Acquired immunodeficiency diseases (HIV, acquired neutropenia)
        - Inflammatory diseases (rheumatoid arthritis, osteoarthritis)
    - Other systemic disorders that influence the pathogenesis of periodontal diseases
        - emotional stress and depression

        - Smoking (nicotine dependence)
        - Medications
    - Systemic disorders that can result in loss of periodontal tissue independent of periodontitis
        - Neoplasms
        - Other disorders that may affect periodontal tissue (Granulomatosis with polyangiitis, Systemic sclerosis (scleroderma), Giant cell granulomas, etc.)

The two major periodontitis subdivisions of the 1999 classification, chronic – and aggressive periodontitis, were entirely replaced by a multidimensional staging and grading system (Table 1 and Table 2) with the emphasis to correct the shortcomings of a lack of clear pathobiology-based distinction between the categories, diagnostic imprecision and problems of implementing the diagnoses in therapy concepts.

- Staging

The staging aspect is defined by severity, complexity and extent and distribution and is subdivided in four rising categories. Severity is described by the level of interproximal CAL, radiographic bone loss (RBL) and tooth loss. Complexity is a modifying factor for staging and may shift the initial stage to a higher level, like furcation II or III involvement, PD ≥6mm, vertical bone loss ≥3mm, secondary occlusal trauma, bite collapse, drifting, flaring, masticatory dysfunction or less than 20 remaining teeth.

The extent and distribution should be added as a descriptor for every stage. Extent is described as localized (if <30% of teeth are involved), generalized or molar/incisor pattern is present.

Stage I		
Severity	Interdental CAL at site of greatest loss:	1 to 2 mm
	RBL:	Coronal third (<15%)
	Tooth loss:	No tooth loss due to periodontitis
Complexity	Local:	Maximum PD ≤4 mm
		Mostly horizontal bone loss
Stage II		
Severity	Interdental CAL at site of greatest loss:	3 to 4 mm
	RBL:	Coronal third (15%-33%)
	Tooth loss:	No tooth loss due to periodontitis
Complexity	Local:	Maximum PD ≤5 mm,
		Mostly horizontal bone loss
Stage III		
Severity	Interdental CAL at site of greatest loss:	≥5 mm
	RBL:	Extending to mid-third of root and beyond
	Tooth loss:	Tooth loss due to periodontitis of ≤4 teeth
Complexity	Local:	In addition to stage II:
		Probing depth ≥6 mm Vertical bone loss≥3 mm Furcation involvement Class II or III Moderate ridge defect
Stage IV		
Severity	Interdental CAL at site of greatest loss:	≥5 mm
	RBL:	Extending to mid-third of root and beyond
	Tooth loss:	Tooth loss due to periodontitis of ≥5 teeth
Complexity	Local:	In addition to stage III:
		Need for complex rehabilitation due Masticatory dysfunction Secondary occlusal trauma Tooth mobility degree ≥ 2 Severe ridge defect Bite collapse, drifting, flaring < than 20 remained teeth or 10 opposing pairs

Table 1. Staging system of Periodontitis

- Grading

The grading is the second important column which is needed to be able to determine a periodontal case classification. Grades A, B and C reflect biologic features of the disease including evidence of or risk for, rapid progression, anticipated treatment response, and effects on systemic health. Grade A reflects a slow, B a moderate and C a rapid rate of progression. Evidence of progression can be found directly through RBL or CAL or indirectly with the ratio of percentage of bone loss and age, or the individual case phenotype with consideration of the amount of biofilm of deposits and the expected destruction of periodontal tissues. The risk factors smoking and glycemic control in diabetes patients serve es grade modifiers that can shift to a higher progression rate if applicable.

Grade A – slow rate of progression		
Primary Criteria	Longitudinal data (RBL or CAL):	Evidence of no loss over 5 years
	% bone loss/age:	< 0.25
	Case phenotype:	↑ biofilm deposits with ↓ levels of destruction
Grade modifiers	Smoking:	non-smoker
	Diabetes:	Normoglycemic / no diagnosis of diabetes
Grade B – moderate rate of progression		
Primary Criteria	Longitudinal data (RBL or CAL):	<2 mm over 5 years
	% bone loss/age:	0.25 to 1
	Case phenotype:	Destruction ≙ biofilm deposits
Grade modifiers	Smoking:	<10 cigarettes per day
	Diabetes:	HbA1c < 7.0% in patients with diabetes
Grade C – severe rate of progression		
Primary Criteria	Longitudinal data (RBL or CAL):	≥2 mm over 5 years
	% bone loss/age:	< 1
	Case phenotype:	↑ Destruction with ↓ biofilm deposits specific clinical patterns suggestive of periods of rapid progression and/or early onset disease e.g.: molar/incisor pattern; lack of expected response to standard bacterial control therapies
Grade modifiers	Smoking:	≥ 10 cigarettes per day
	Diabetes:	HbA1c ≥ 7.0% in patients with diabetes

Table 2. Grading system of Periodontitis

iv. **Prevalence**

Periodontitis is a very common disease(Marcenes et al., 2013) and affects 30% to 50% of adults in the United States(American Academy of Periodontology, 2005) comparable to European countries like Germany(Jordan et al., 2014) and Denmark(Krustrup & Erik Petersen, 2006) with a higher prevalence in developing countries(Pihlstrom, Michalowicz, & Johnson, 2005). Periodontitis patients are often not presenting any pain symptoms. This also a bit loosely called "silent disease" with bleeding and swelling of the gums, tooth mobility and halitosis can exist unnoticed for several decades and can impair the individual well-being and quality of life. The American Dental Association (ADA) published a resolution stating that "oral health is a functional, structural, aesthetic, physiological and psychological state of well-being and is essential to an individual's general health and quality of life"(Glick & Meyer, 2014). Periodontitis has not only negative influences on the oral cavity, it is also a medical and socioeconomical burden.

v. **Periodontal Medicine and Systemic Diseases**

In 1900, William Hunter considers that there is a wide field of preventive medicine open by the exercise of oral antisepsis(Hunter, 1900). He reported, with the help of some of his own clinical cases, that pus organisms from the mouth may contribute to systemic conditions like intestinal troubles, nervous attacks, gastritis and curious rashes. He comes to the insight, that no physician or surgeon would tolerate a foul septic ulcer in the forearm, but that this would be exactly the case with patients with necrosed teeth and stomatitis.

Until 30 years ago, the effect of periodontal disease on general health was more or less narrative, based on empirical evidence. With the upcoming of better understanding the pathophysiological pathways and the conduct of preliminary and advanced clinical studies emerging evidence of associations between the periodontal status and systemic health lead to the development of a new branch in periodontology, the periodontal medicine (Williams & Offenbacher, 2000). This terminus describes the "two-way relationship which periodontal disease in an individual may be a powerful influence on an individual's systemic health or disease as well as the more customarily understood role that systemic disease may have in influencing an individual's periodontal health or disease". Periodontal diseases have impact on general health and are known to negatively influence systemic health and/or conditions for instance diabetes, cardiovascular disease or nosocomial pneumonia(Gomes-Filho et al., 2014).

vi. Periodontitis and cardiovascular diseases (CVD)

In the mid 90's of the former century the first hypotheses were made to show, that periodontitis is an unrecognized risk factor for atherosclerosis and thromboembolic events (Beck, James, Garcia, Heiss, Vokonas, & Offenbacher, 1996). The mechanisms were explained on the basis, that an underlying inflammatory mechanism is common in periodontal disease and atherosclerosis. Periodontitis contributes to an increase of metastasizing lipopolysaccharides and cytokines like Interleukin -1β (IL-1 β), Prostaglandin E2 (PGE2) and tumor necrosis factor - α (TNF- α).

vii. Risk factors for Periodontal disease and cardiovascular diseases

Local, systemic, genetic and/or environmental factors can contribute to the development of periodontal disease and cardiovascular diseases. In a "balanced" oral microflora, inflammatory processes, as a reaction to pathogenic microorganisms, often take place without loss of attachment. Several risk-factors are known to shift this process into a more aggressive and destructive host immune response. Poor oral hygiene, smoking, uncontrolled diabetes mellitus, osteoporosis, rheumatoid arthritis, obesity, stress and poor coping behaviors are the most known and dangerous risk factors for periodontal disease. Gene-polymorphisms also have been associated with the easier development for periodontal disease.

Risk factors for CVD are often similar and have a rather complex interaction with the host immune response. Some of them can't be changed, like age, history of CVD in the family, race or genetic predisposing factors. A large proportion of risk factors are environmental or better said behavioral. They can be modified and are often responsible for a large number of other unhealthy systemic conditions. These changeable factors are i.e. smoking, heavy body weight and obesity, low physical activity and diabetes mellitus. They are also triggering factors that can lead to CVD pathologies like plaque formation, rupture and thrombosis, which can lead to non-fatal or fatal cardiovascular endpoints like myocardial infarction, cerebrovascular insult or death.

Tobacco smoking certainly is the major behavioral risk-factor for the development and progression of periodontal disease and CVD. Helping patients on an individual level in explicitly highlighting the positive effects of smoking cessation and informing them about specific programs is of utmost importance.

viii. **Cigarettes smoking as a risk factor for periodontal disease**

In 2017, the World Health Organization (WHO) reports that every year seven million people die from using tobacco. It is also very well known that industrial processed tobacco contains over 7000 toxic chemicals, many of them are perfectly known and associated to be carcinogen (World Health Organization, 2017) and stopping smoking is the most important key steps in cancer prevention today. According to Eurostat (https://appsso.eurostat.ec.europa.eu/nui/submitViewTableAction.do 01.12.2019) in the year 2014 approximately 23.9% of the population of Austria were daily smokers of cigarettes. The average percentage of smokers in the 28 countries of the European Union was 18.4% at the same time.

Tobacco smoking is by far the most important environmental risk factor in periodontitis. The nicotine addiction is so high, that no other drug is used so frequently in such a short period of time and the negative effects come from long term exposure and not from the single use (Palmer, Wilson, Hasan, & Scott, 2005). This has to be taken into consideration when explaining to smoking patients the negative effects of tobacco use and makes clear why punctual or half-hearted attempts to show the beneficial sides of smoking cessation are often doomed to failure.

Smoking affects not only directly the oral and respiratory tissues, it has also negative effects on dental biofilm, inflammatory- and host immune response (Tonetti, Maurizio S., 1998). Smokers have similar plaque-formation rates like non – smokers but smokers have a suppressed inflammatory response to plaque-formation than non-smokers (Lie, Timmerman, Van der Velden, & Van der Weijden, 1998). It could not be shown specifically that the micro-flora in smokers and non-smokers differ a lot from each other in oral location (Mager, Haffajee, & Socransky, 2003) but the infection rate seems to be higher in smokers (Shiloah, Patters, & Waring, 2000). Concerning the subgingival flora and evaluation based on PCR technologies, in periodontitis patients, there is some evidence that smokers have a higher prevalence of putative periodontal pathogenic bacteria like Tannerella forsythia or Porphyromonas gingivalis(Darby, Hodge, Riggio, & Kinane, 2000). More recent findings on the basis of 16S rRNA gene sequencing methods indicate that smoking contributes to a more anaerobic oral environment and a bacterial community with reduced xenobiotic degradation capabilities (Wu et al., 2016)

In addition to an altered oral microbiome and a higher susceptibility to the occurrence of periodontal diseases, the symptoms are often concealed by the fact that the classical

response to inflammation in periodontal tissues, bleeding, is lower in smokers compared to non-smokers with the same disease progression (Dietrich, Bernimoulin, & Glynn, 2004).

ix. Pathogenic Mechanisms between Periodontal Disease and CVD

Two pathways have been described linking Periodontal Disease and CVD; direct and indirect pathomechanisms (Lockhart et al., 2012).

1. Direct mechanism

Bacteremia is the key mechanism for direct influence of the pathogens, that the oral cavity harbours. The periodontal pocket is a perfect location for biofilm growth, organization and differentiation. It is not surprising, that during regular habits and procedures like chewing and tooth brushing, a large number of bacteria can enter the bloodstream and can be identified there. Periodontal pathogens can invade human tissues like vascular cells. At various sites microorganisms of oral origin can colonize the tissue surface and/or penetrate them. In example on the heart valve and in atheromatous plaque a high percentage of cariogenic streptococcus mutans bacteria have been found (Nakashima, Plump, Raines, Breslow, & Ross, 1994). Detection of viable oral bacteria via Polymerase Chain Reaction (PCR) techniqes in atheromas has not been shown but DNA of periodontal pathogens is detectable in atherosclerotic plaques (Fiehn, Larsen, Christiansen, Holmstrup, & Schroeder, 2005) . Other bacteria species are common in carotid atheromas as well, even if DNA for periodontal – pathogens is not always found or tested positive when species-specific primers for their detection have been used (Aimetti, Romano, & Nessi, 2007).

2. Indirect mechanism

The evolution and initialization of atherosclerotic plaque is usually a chronic, slow process of growth. If unstable, these plaques can rupture and release dangerous thrombogenic components, which can be causative for myocardial infarct, acute coronary syndrome or stroke(Falk, Shah, & Fuster, 1995). Systemic inflammatory components and processes contribute to growing, thinning and therefore more vulnerability of the atheromatous plaque. Higher systemic inflammatory marker, like the unspecific CRP, can be measured even in periodontal inflammation. Other inflammatory markers like TNF-α, IL-1 and Interleukin-6 (IL-6) also are found to be higher in periodontitis patients than in healthy individuals (Loos, Craandijk, Hoek, Wertheim-van Dillen, & Van Der Velden, 2000; Noack et al., 2001).

Although the evidence for supporting the theory, that pro-inflammatory mediators play this special role in increasing the risk for CVD events with concomitant periodontitis, is not very strong (Teles & Wang, 2011) the mechanisms are biological plausible. "*These mediators from the periodontium could affect other organs, such as the liver, to initiate an acute-phase response that would impact other organs. This would lead to inflammatory changes in the endothelium such as up-regulation of adhesion molecules and promotion of cytokine production, and thus initiation or acceleration of atheroma development*(Schenkein & Loos, 2013b).

b. PERIPHERAL ARTERIAL DISEASE

i. Definition and Epidemiology

Peripheral arterial disease is the third most common clinical manifestation of atherosclerosis after coronary artery disease and stroke (Fowkes, F. Gerald R. et al., 2013). Peripheral arterial diseases (PAD) is the umbrella term for diseases of non-coronary arteries. Synonyms used are peripheral vascular disease, peripheral arterial occlusive disease, and lower extremity arterial disease. PAD includes atherosclerosis, plaque rupture, abnormal vascular reactivity, vasospasm, inflammation, arterial wall dysplasia and thrombus formation leading to occlusion (Mohler, Jaff., 2017) and over 200 million people have PAD worldwide. There are also estimations, that for every 100 patients with intermittent claudication (IC) there are 200 with asymptomatic or untreated PAD and therefore a serious disease with possible serious outcomes (Dormandy, Heeck, & Vig, 1999).

ii. Prevalence

The prevalence in younger populations is quite low and raises with age (Fowkes, F. Gerry R. et al., 2017) and affects approximately over 20% of patients older than 75 years of age (Diehm et al., 2004) .

iii. Symptoms

Insufficient blood supply to the lower extremities can lead to pain known as IC. The painful episodes occur with walking and find relief when the patient rests. The assessment of the symptoms can be done by validated questionnaires, like the San Diego Claudication Questionnaire. However, there are patients reporting no pain symptoms, also in moderate to severe cases. Differences in intensity, like pain beginning at rest or pain-free cases, which can be due to patient's high activity level, have been reported (Criqui et al., 1996). There are also comorbidities reported, such as hip and knee arthritis, back disease or neuropathy, which can change the individual pain symptoms in PAD (McDermott, Mary McGrae et al., 2001). The lack of objectivity in questionnaires is a factor of uncertainty and could be the source of false- or under-estimation of leg pain. Since the 1950's the measurement of blood pressure at the ankle was used for evaluation of PAD and the ankle-brachial index (ABI) developed.

iv. **Ankle-brachial index (ABI)**

The ABI is the ratio of the systolic blood pressure at the ankle to that in the arm. It is a specific and sensitive non-invasive test for PAD diagnosis. But even in absence of PAD symptoms, the ABI is an indicator of atherosclerosis apart from the lower extremities and can serve as prognostic marker for cardiovascular events and functional impairment (Criqui et al., 1992) (McDermott, Mary M. et al., 2009) (Ankle Brachial Index Collaboration et al., 2008). Cardiovascular societies around the world recommend the measurement of the ABI in smokers and diabetic patients over 50 years and everyone else over 70 years. Low values of ABI are characteristic for atherosclerosis of the lower extremities. ABI of ≤0.90 is used to diagnose symptomatic or asymptomatic PAD.

v. **Classification**

One of the most common clinical classification systems is the Fontaine's one(Fontaine, 1954) . It is divided into four stages, where subjective symptoms, objective signs, investigational and laboratory findings are considered as shown in Table 3 and 4 [cited from (Novo, 2002)].

Another very comprehensive classification is the Rutherford Classification(Rutherford et al., 1986; Rutherford et al., 1997) which differentiates into acute and chronic limb ischemia with different treatment plans. The classification is very similar to Fontaine's staging with the addition of objective data. Table 5 and 6 (Hardman, Jazaeri, Yi, Smith, & Gupta, 2014) show an overview of the classification for chronic and acute limb ischemia.

	Stage I	Stage II	Stage III	Stage IV
Subjective symptoms				
Paraesthesia	+ –	+ +	+ + +	+ + +
Cold extremities	+ –	+ +	+ + +	+ + +
Pain	no	IC	rest pain	rest pain
Objectives signs				
Pulselessness	+ –	+ +	+ + +	+ + + +
Vascular bruits	+	+ +	+ + +	+ + +
Hair loss	+ –	+	+ +	+ + +
Trophic alterations of nails	no	+ –	+ +	+ + +
Muscular hypotrophy	no	+	+ +	+ + +
↓ Tissue temperature	no	+ –	+ +	+ + +
Trophic lesions	no	no	no	yes

Table 3. Subjective symptoms and objective signs in peripheral arterial disease

Examination	Stage I	Stage II	Stage III	Stage IV
↓ ABP Index (Doppler)	+	+ +	+ + +	+ + + +
↓ Peak flow (Plethysmography)	+	+ +	+ + +	+ + + +
Peak flow/Rest flow	+	+ +	+ + +	+ + + +
Doppler alterations	+	+ +	+ + +	+ + + +
Echo-Doppler alterations	+	+ +	+ + +	+ + + +
Laser-Doppler alterations	no	+ –	+ +	+ + +
Capillaroscopy alterations	no	no	+	+ + +
↓TcO_2	no	no	+ +	+ + +
Whole blood viscosity	+ –	+	+ +	+ + +
Red cells deformability	+ –	+	+ +	+ + +
↑ Fibrinogen	+ –	+	+ +	+ + +
↑ Platelet function	+ –	+	+ +	+ + +
Coagulation activation	+ –	+	+ +	+ + +
Fibrinolysis inhibition	+ –	+	+ +	+ + +

Table 4. Investigational and laboratory findings in peripheral arterial disease

Grade	Category	Clinical description	Objective criteria
0	0	Asymptomatic—no hemodynamically significant occlusive disease	Normal treadmill or reactive hyperemia test
	1	Mild claudication	Completes treadmill exercise; AP after exercise > 50 mm Hg but at least 20 mm Hg lower than resting value
I	2	Moderate claudication	Between categories 1 and 3
	3	Severe claudication	Cannot complete standard treadmill exercise, and AP after exercise < 50 mm Hg
II	4	Ischemic rest pain	Resting AP < 40 mm Hg, flat or barely pulsatile ankle or metatarsal PVR; TP < 30 mm Hg
III	5	Minor tissue loss—nonhealing ulcer, focal gangrene with diffuse pedal ischemia	Resting AP < 60 mm Hg, ankle or metatarsal PVR flat or barely pulsatile; TP < 40 mm Hg
	6	Major tissue loss—extending above TM level, functional foot no longer salvageable	Same as category 5

Table 5. Rutherford classification for chronic limb ischemia

Category	Description/Prognosis	Findings		Doppler signal	
		Sensory loss	Muscle weakness	Arterial	Venous
I. Viable	Not immediately threatened	None	None	Audible	Audible
II. Threatened					
a. Marginally	Salvageable if promptly treated	Minimal (toes) or none	None	Inaudible	Audible
b. Immediately	Salvageable with immediate revascularization	More than toes, associated rest pain	Mild, moderate	Inaudible	Audible
III. Irreversible	Major tissue loss or permanent nerve damage inevitable	Profound, anesthetic	Profound, paralysis	Inaudible	Inaudible

Table 6. Rutherford classification for acute limb ischemia

vi. **Association between PAD and Periodontitis**

The mechanisms underlying the association between periodontitis and PAD are not yet fully understood. As already mentioned, elevated inflammatory mediators like IL-6, IL-1β and TNF-α in systemic circulation within PAD patients suggests that chronic infection in the body may play an important role (Chen et al., 2008).

The first reports showing that periodontitis patients have a 2.27-fold higher risk for developing PAD were published in the late 90's of the last century (Mendez et al., 1998). A recent meta – analysis evaluated the possible links between PAD and periodontal diseases. Seven studies were included with high heterogeneity (case – control, cross – sectional and prospective cohort). A significant association of increased risk of periodontitis in PAD patients compared to healthy participants could be shown (Yang et al., 2018). Additionally, the number of missing teeth, which can reflect end – stage periodontal conditions (Hyde, Dupuis, Mariri, & Dartevelle, 2017), was higher in PAD patients. However, there are rare studies, that in addition have significant differences in assessment of disease and number of patients. There's definitively a lack of epidemiological studies to assess the prevalence of periodontitis in PAD patients. CAL and number of missing teeth should be taken as one of the main parameters in clinical, high - quality studies.

Even rarer are interventional studies in patients with periodontitis and concomitant PAD. The Periodontitis and Vascular Events (PAVE) pilot study showed, that it is feasible to investigate the influence of periodontal therapy vascular parameters and clinical outcomes (Couper et al., 2008). Preliminary studies (Seinost et al., 2005b) (Tonetti, Maurizio S., D'Aiuto, Nibali, Donald, Storry, Parkar, Suvan, Hingorani, Vallance, & Deanfield, 2007b) showed, that there is an influence by periodontal therapy on endothelial function.

There are no prospective periodontal interventional studies on the primary prevention of CVD (first ischaemic events or cardiovascular death) and it has been questioned if it's even feasible to perform adequate randomized controlled trials due to ethical, methodological and financial considerations (Sanz et al., 2020; Tonetti, Maurizio S. & Van Dyke, 2013). The Atherosclerosis Risk in Communities (ARIC) study assessed 6736 dentate subjects for periodontal disease status. Periodontitis was significantly associated with cardioembolic and thrombotic stroke subtypes. Patients with regular dental care utilization and follow ups had a lower adjusted stroke risk (Sen et al., 2018). A large population based study with 247 696 healthy adults of 40 years or older with no history of major cardiovascular events showed that patients who underwent regular dental check – ups and professional cleaning had a lower risk for developing cardiovascular events

(follow – up of 9.5 years) than patients with periodontal disease, caries and higher number of missing teeth. (Park et al., 2019).

The impact of periodontal treatment on general health, especially on CVD is still a matter of debate. Therefore, this work aims to collect more epidemiological data and to evaluate the influence of standard non-surgical therapy on patients with PAD.

4. METHODS

a. Study Design

This investigation is a sub study of the PeriPAD trial, a single – centre, prospective, randomized, open trial conducted at the Department of Internal Medicine, Division of Angiology and at the Department of Dental Medicine and Oral Health, Division of Restorative Dentistry, Periodontology and Prosthodontics, Medical University Graz, Austria, to investigate the influence of periodontal therapy on vascular inflammation and function in patients with peripheral arterial disease. PeriPAD was registered as a randomized controlled trial at the DRKS (Deutsches Register Klinischer Studien; https://drks-neu.uniklinik-freiburg.de/drks_web/) ID: 00004554. Ethical approval granted by the Ethics Committee of the Medical University of Graz, Austria (EK-Nr. 24-456 ex 11/12). The study was funded by the FWF and performed in full accordance with the requirements of the Declaration of Helsinki and the Good Clinical Practice Guideline. All participants included in the study gave written informed consent before participation.

Consecutive patients from the Division of Angiology with symptomatic PAD were screened for the presence for periodontitis. Patients were recruited from the outpatient clinic and inpatient ward of the Division of Angiology as well as from the outpatient clinic of the Department of Vascular Surgery at the Medical University Graz. Communication with the participants was done by a study co-working colleague either via telephone or in person and the screening was performed by her in the morning after an overnight fast at the outpatient clinic for preventive vascular medicine at the Department of Angiology. Patients who had recently undergone a percutaneous transluminal angioplasty were scheduled for a screening appointment at least 2 weeks after hospital discharge to avoid any influence of inflammatory processes resulting from the endovascular intervention. Patients were sent for dental examination, if at least one natural tooth was still in the mouth.

A total of 414 screening patients were sent to the Department of Dental Medicine and Oral Health after examination from the Department of Angiology from February 2013 until December 2015. Of the 414 screening patients 5 did not show up at the Department of Dental Medicine and Oral Health without any known reason. The remaining 409 screening patients were examined at the Division of Restorative Dentistry, Periodontology and Prosthodontics by one single examiner. 296 screening patients were male and 113 were female. They were aged between 39 and 89 years with a mean age of 63 years.

Following inclusion criteria were defined:

- Symptomatic PAD
    - PAD **Fontaine's stages II** and documented **luminal stenosis >70%** on ultrasound or angiography
    - PAD **Fontaine's stages III** and documented **luminal stenosis >70%** on ultrasound or angiography
- Periodontal inclusion criteria
    - presence of at least **twelve natural teeth**, including third molars
    - at least two teeth with **probing depth >5mm**
    - at least two teeth with **clinical attachment loss >5mm**
    - and **>20%** of sites having **bleeding on probing**
- Signed informed consent

Following exclusion criteria were defined:

- PAD Fontaine's stage IV (tissue damage/loss)
- Life expectancy <6 months
- Instable cerebrovascular and cardiovascular disease
- Clinically apparent infectious disease
    - pneumonia
    - symptomatic urinary tract infection
- Systemic inflammatory disease
    - chronic inflammatory bowel disease
    - rheumatoid arthritis
    - vasculitis by clinical assessment
- Periodontal treatment within 6 months of the study
- Mouth infection other than periodontitis
- Uncontrolled diabetes
- Pregnancy

- Age <18 years
- Consumption of drugs known to affect periodontal status
    - anticonvulsants
    - immunosuppressants
- Allergy to penicillin and/or metronidazole

The screening process stopped as the aspired number of 90 study patients was reached. They were eligible to be enrolled in the study and were randomized into one of three groups, two of them involving non-surgical periodontal treatment:

Periodontal Therapy Group 1 (PT1): patients received periodontal therapy including the use of antibiotics.

Periodontal Therapy Group 2 (PT2): patients received periodontal therapy without antibiotics.

Control Group 3 (CG): was designed as control group with patients receiving a tentative diagnosis and instructions but no specific therapy for the first 3 months of the study period.

This dissertation is subdivided in two parts. The first part is concentrating on the prevalence of periodontal diseases in the screening cohort. The second part deals with the question of the effectiveness and influences of different non-surgical periodontal treatment concepts and no treatment compared to each other.

i. In – vitro study of surface changes induced to enamel and cementum by different scaling and polishing techniques

Parallel to the clinical study we wanted to evaluate how the most common techniques of scaling and root planing affect the surface roughness and substance thickness of dental hard tissues (enamel and cementum). We also wanted to assess the surface qualities after additional cleaning with polishing pastes in decreasing abrasiveness. Therefore, we subjected five impacted third molars after extraction to a total of nine treatment groups. Air – polishing with non – abrasive erythritol powders with 7µm particle size, Ultrasonic scaling with piezo – electrical devices and hand instruments (scalers and curettes). All three techniques have been combined and in the end rubber – cup paste polishing was performed. Thorough rinsing with sterile saline solution was performed after every procedure. A non – contact optical scanning system (Infinite Focus G5; Alicona Graz, Austria) measured surface roughness and substance loss.

b. Baseline Examination

Patients presenting to the Department of Dental Medicine and Oral Health were asked to fill out a general medical and dental history form. An orthopantomogram was performed if none was performed and available during the last 6 months at the Department of Dental Medicine and Oral Health.

An evaluation of other dental infections and caries was performed using the Decayed, Missing, Filled, Teeth (DMFT) index. In addition to the DMFT, the number of remaining teeth, including dental implants and non-retained third molars was evaluated.

A standardized periodontal screening method, using the PGU ("Parodontale Grunduntersuchung") score system, which is based on the Community Periodontal Treatment of Index Needs (CPITN)(Ainamo, 1982) was used to categorize the patients into three groups: periodontal healthy (PGU 0), moderate (PGU 1) and severe gingivitis (PGU 2), moderate (PGU3) and severe periodontitis (PGU 4). This grading system is equivalent to the PSR (Periodontal Screening and Recording) index, using a WHO Perio-Probe.

The highest grade was recorded for each quadrant and the patients were categorized with their highest PGU grade respectively. If a severe dental infection was present the participants were asked to see their dentists or make an appointment at the Department for Dental Medicine and Oral Health to get the appropriate treatment. If it would have occurred, those patients would have been able to participate to the study.

c. Intervention

Screening patients who met the inclusion criteria were eligible to be included in the study and were asked to attend another visit at the Department of Angiology, where they were randomized into one of the three patient groups described above (PT1, PT2, and CG). Randomization was performed using a computerized randomizer tool (https://www.randomizer.at/).

After randomization to one of the three study groups the patients were treated as followed: 30 patients were allocated to a non-surgical periodontal treatment with adjuvant systemic

antibiotics group (PT 1), 30 patients received a non-surgical periodontal treatment (PT 2) and 30 patients received no treatment (Control group). Full-mouth periodontal status was done in every group using a computerized periodontal probe with 1-mm markings, Florida Probe® (Florida Probe Corp., Gainesville, FL, USA) (Gibbs et al., 1988). Overview of the different study groups and interventions is given in Figure 1.

BOP is the key parameter for ongoing inflammation of the gums and the teeth supporting tissues. But even with calibrated periodontal probes there were several factors which lead to non-reliable PD and/or CAL measurements. In these cases, a manual probing was done to reassure the measurements, especially if calculus, restorative margins, differents in dental implant designs and supra – structures were given and would have been sources of errors and non-comparable values. However today the combination of PD and BOP seems to fit best to reflect the periodontal inflammatory burden.

The Periodontal inflamed surface area (PISA) classification quantifies the amount of inflamed periodontal tissues by reflecting the surface area of bleeding pocket epithelium(Nesse et al., 2008). Nesse et al. constructed a Microsoft Excel spreadsheet to facilitate the determination of the inflamed surface area in millimetres square, which can be downloaded for free at https://www.parsprototo.info/pisa.html.

Non-surgical treatment groups patients (PT1 and PT2) underwent a session of supragingival scaling and polishing and received instruction on how to perform proper plaque control methods. These initial steps have been done by one single experienced clinician. At the end of the session, baseline periodontal clinical measurements (PD, mobility, furcation involvement, recessions) were recorded by a single blinded dental prophylaxis assistant except for both plaque index and bleeding index that were assessed immediately prior to scaling (Gibbs et al., 1988). Within one week the study patients from groups PT1 and PT2 were scheduled for one-stage-full-mouth disinfection (OSFMD) according to a standardized protocol (Quirynen et al., 1999). Full-mouth subgingival scaling and root planing (SRP) was performed in maximum two sessions within 24 hours by a single experienced therapist using hand instruments and ultrasonic scalers. Subgingival instrumentation was performed under local anaesthesia without a time limit until the root surface felt smooth and clean to an explorer tip. Mechanical debridement was supplemented by disinfection of intra-oral niches. Immediately after each instrumentation session, the dorsum of the tongue was brushed by the patient with a 1% chlorhexidine (CHX) gel for 1 min. The mouth was rinsed twice with a 0.2% CHX solution for 1 min, the pharynx was sprayed with a 0.2% CHX spray and all pockets were irrigated (three times

within 10 min.) with a 1% CHX gel. Subjects were instructed to use a 0.2% CHX rinse twice daily for 1 min for 2 months post-treatment.

The CHX rinses started with the first session of OSFMD. Patients in the PT1 group additionally received systemic antibiotics (amoxicillin 500 mg plus clavulanic acid 125mg and metronidazole 500 mg). They were advised to take both antibiotics three times a day for 7 days (Yek et al. 2010). During the post-treatment phase until follow up the patients' oral hygiene standards was reviewed and oral hygiene procedures were reinforced.

For reassessment examinations full-mouth clinical examination was performed again by the same blinded dental prophylaxis assistant at 3 months and 12 months after the first study visit. Clinical, microbial and immunological periodontal parameters assessed at baseline were recorded again.

Participants randomized to the CG were given a copy of their panoramic x-ray and a letter stating the tentative diagnosis. Full-mouth periodontal status was done using a computerized periodontal probe. They received dental hygiene instructions and were encouraged not to see a dentist for the study period of 3 months, except in cases of emergency. Periodontal reassessment examinations were performed at 3 months and 12 months.

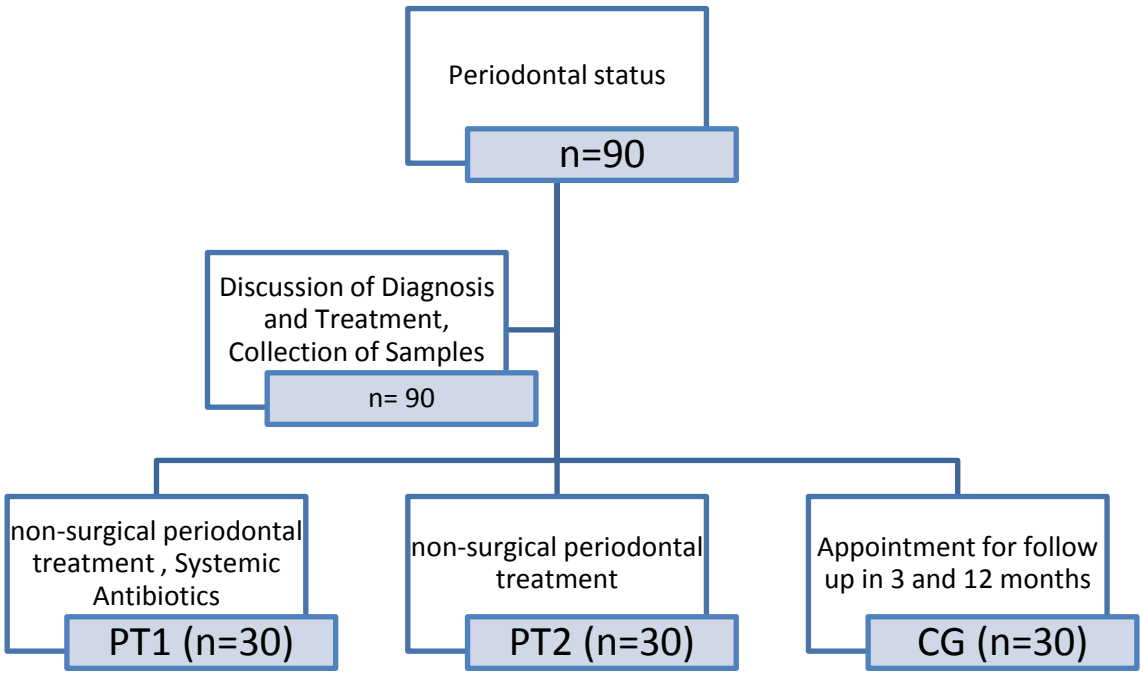

Figure 1: Intervention(PT1 and PT2)/Control group (CG)

d. Collection of subgingival plaque

At the baseline study visit and the two follow up visits, 3 months and 12 months after initial presentation, subgingival plaque samples were collected from the study patients. For this specific gathering of biological samples, a separate informed consent was obtained by the study participants. They gave explicit permission, that the biomaterial can be stored for later microbiological analysis.

Before collection of subgingival plaque, the supragingival surfaces was cleaned using sterile cotton pellets to minimize the mixture of supra- and subgingival biomaterial.

The biofilm samples were obtained at the four periodontally most affected teeth in every quadrant using matching sterile Gracey curettes (Deppeler™) 5/6, 7/8, 11/12 and 13/14 for the incisors/canines, premolar/molar buccal sites, premolar/molar medial sites and premolar/molar distal sites respectively. The recovered subgingival biomaterial was transferred to a sterile test tube which contains 100µl Tris(hydroxymethyl)-aminomethan Ethylenediaminetetraacetic acid (TE) of a buffer solution.

This procedure was performed before 8 weeks and 9-10 months after the last session of periodontal treatment. Patients in the Community dental care group underwent the procedure before, 3 and 12 months after the first acquisition of biofilm samples.

The pooled sample tubes were put into dry ice immediately for transportation to a freezer, where the samples were stored at a constant temperature of -80° Celsius. The sample storage racks (Micronic Roborack-96) were completely filled, holding 96 individual tubes (8x12) in a 96-well configuration and were stored at the Department of Dental Medicine and Oral Health.

After collecting all samples (baseline, follow-up I and follow-up II) the molecular biological analysis was performed at the center for medical Research at the Medical University of Graz using “The Genome Sequencer FLX System”, which is one of the so–called "Full–Genome Sequencing Systems". Up to 1.3 Mio reads with an average length of ~ 400bp can be generated within a single run (~ 500.000.000 bases in less than 24 hrs). These characteristics predispose the FLX system for various applications in the field of high–throughput sequencing.

Sequencing of the obtained oral subgingival plaque samples was performed at the Centre for medical Research (ZMF) Graz at the Molecular Biology Core Facility, which offers infrastructure and expert technical support for the successful application of all key molecular biology techniques.

Bioinformatical analysis of data from the "Next Generation Sequencing" was performed by the office of bioinformatics at the ZMF Graz. Biostatistical service including collection, examination, analysis, presentation and interpretation of oral microbiological data was provided by the office for biostatistics at the ZMF Graz.

e. Collection of Matrix-metalloproteinase-8

Neutrophil collagenase or collagenase-2 (matrix metalloproteinase [MMP]-8) belongs to the collagenase subgroup of the MMP superfamily of calcium- and zinc-dependent neutral proteinases (Sorsa et al., 2011). Matrix-metalloproteinase-8 (MMP-8) is one of the key enzymes in catabolic mechanisms, leading to disintegration of collagen in periodontal tissues. It is also one of the most examined and used biomarker in gingival crevicular fluid (GCF).

Methods for collecting MMP-8 for a biomarker test followed a standardized protocol. Care was taken, that no mechanical cleaning or probing was taken out prior to collection of GCF. Bleeding in the sulcus was tried to be avoided before the strips were inserted. Sulcus fluid samples were taken with sterile paper strips from the four highest probing depths of all 4 quadrants. If one quadrant was edentulous, the two deepest sites were collected in the same jaw. Before the insertion of the strip, the corresponding site was dried selectively with air to remove saliva from the site.

The strip was taken sterile from the wearer in the test kit with a sterile dental tweezer at the waxed end. With the other end, the strip was inserted into the sulcus up to the bottom of the pocket and left for about 30 seconds. The strips were then transferred into an eprouvette (pooled collection of GCF) and closed.

At both examination times, sulcus fluid samples were taken using sterile strips in the test kit (dentoELISA aMMP-8) and again sent to the evaluating laboratory (Dentognostics GmbH, Jena - Germany) by air mail, anonymized and contamination-free.

The dentoELISA aMMP-8 is an enzyme immunoassay for the quantitative detection of active matrix metalloproteinase-8 (aMMP-8 = collagenase 2) in sulcular fluid (Gingival Crevicular Fluid = GCF or peri – implant sulcular fluid = PISF) of dental pockets. Sulcus fluid (GCF/ PISF) is analysed as a sample. This is taken with GCF / PISF strips and sent to the laboratory in the sample shipping tube. Specific antibodies against the activated matrix metalloproteinase-8 are fixed in the wells of the microtitration plate. After addition of the patient sample, the aMMP-8 in the sulcus fluid forms immune complexes with the fixed antibodies. Unbound components from the sample incubation are removed by washing processes. A peroxidase-enzyme conjugate is then added which binds to the fixed immune complexes. Unbound components are again removed by washing processes. After addition of the substrate solution, the peroxidase activity produces a blue dye which

changes to yellow after addition of a stop solution. The intensity of the colour is proportional to the concentration of aMMP-8 in the sample.

The concentrations of the aMMP-8 were documented for each patient in ng/ml sulcus fluid in tabular form using Microsoft Excel 2016. Thus, the mean concentration of aMMP-8 in the sulcus of each patient was examined at three time points, baseline after 3 months and 12 months in every study group.

f. Detection of periodontopathogenic bacteria by molecular genetic method – Polymerase chain reaction (PCR)

Collection of microbiological samples were obtained baseline, after 3 months and after 12 months. The samples for PCR testing were taken with sterile paper strips from the four highest probing depths of all 4 quadrants. The sample area was isolated from saliva with cotton rolls and the supragingival surface was gently air – dried. Supragingival plaque was gently cleaned with cotton pellets. The sterile paper strips were inserted in the corresponding pockets for 20 seconds and after removal transferred to a sterile screw capped – tubes. The pooled samples in the tubes were sent to the microbiological laboratory of the institute for hospital hygiene and microbiology, State Hospital Graz, Austria for further analysis.

The micro-IDent® Plus test can identify eleven periodonto – pathogenic bacteria types: Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis Tannerella forsythensis, Treponema denticola, Prevotella intermedia, Peptostreptococcus micros, Fusobacterium nucleatum/periodonticum, Campylobacter rectus, Eubacterium nodatum, Eikenella corrodens and Capnocytophaga species (gingivalis, ochracea, sputigena).

i. PCR

The subgingival plaque samples collected with sterile paper points were used as starting material for DNA extraction. The paper points were placed in a 1.5 ml screw cap tube. According to the manufacturers (HAIN Lifescience GmbH, Nehren, Germany) instructions of use, the paper points of each patient were used for performing even the micro-IDent or the micro-IDent plus 11 tests. Both tests are valid testing methods and have been described and used in a large number of in vitro and in vivo studies.

The laboratory work load and analytical steps as stated below for the institute for hospital hygiene and microbiology are similar to the manufacturer's instructions, which have been described step by step from Urbàn et al. in a reference publication in 2010.

"The micro-IDent is able to identify five key periodontopathogenic bacteria: Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia and Treponema denticola, while the micro-Ident plus 11 test can detect some other putative pathogens in this disease: Parvimonas micra, Fusobacterium nucleatum/p, Campylobacter rectus, Eubacterium nodatum, Eikenella corrodens and Capnocytophaga sp., respectively. These two different commercially available tests are based on the same methods but detect different taxa and consist of 2 distinct techniques i.e. PCR and hybridization with species/genus specific probes." (Urbán, Terhes, Radnai, Gorzó, & Nagy, 2010).

The samples were warmed up to room temperature and the DNA was extracted as follows: 200 µl of Chelex-solution were added to every sample and mixed, after the samples were sonificated for 15 Minutes at 70°C they were put on a heating block at 95°C for 15 minutes. The lysate was vortexed for 30 seconds and spun down for 1 minute at maxium speed in a standard table – top centrifuge. PCR amplification was carried out in a reaction volume of 50 µl consisting of 5 µl of template DNA and 45 µl reaction mixture containing 10 µl of Mix A (primer-nucleotide mix) and 35 µl Mix B containing 10X PCR buffer, 25 mMMgCl2 and 1U Taq polymerase. PCR cycling was carried out in a FlexCycler thermal cycler (Analytic jena, Jena, Germany). The cycling conditions comprised an initial denaturation step at 95 C for 15 min, 10 cycles at 95 C for 30 s and at 58 C for 2 min, 20 cycles at 95 C for 25 s, at 53 C for 40 s and 70 C for 40 s and a final extension step at 70 C for 8 min.

Likewise, all steps as mentioned by the manufacturer's recommendations and published by Urbàn et al. have been performed for the subsequent hybridization: The biotin-labelled amplicons were denatured and incubated at 45 C for 30 min with hybridization buffer. Each strip coated with two control lines and five or six species-specific probes, respectively. The first control is a conjugate control, which can demonstrate the efficiency of conjugate binding and substrate reaction. The second control, namely amplification control can detect the successful amplification. After PCR products had bound to their respective complementary probe, a highly specific washing step removed any non-specifically bound DNA. Streptavidin conjugated alkaline phosphatase was added, strips were washed and hybridization products were visualized by adding substrate concentrate containing dimethyl-sulfoxide... According to the manufacturer, the cut-off of the test is set

to 103 to104 genome equivalents." The DNA-based analysis and identification procedures were performed by two separate, blinded examiners (Urbán et al., 2010) .

g. Periodontal Susceptibility Test (PST)

The IL-1 genotype is discussed to have a role in the pathogenesis and clinical course of periodontitis(Kornman et al., 1997). Testing for evaluation for IL-1 gene – polymorphisms in all included study patients was performed baseline at the first study visit. A sterile cotton swab was used on the inner cheek oral mucosa for 30 seconds to gain sufficient cell counts for the laboratory test. The swabs were transferred to the corresponding testing tubes and sent to the institute for hospital hygiene and microbiology, State Hospital Graz, Austria for further analysis. Genotyping was performed without knowing the periodontal diagnosis. Following single nucleotide variations have been tested: IL-1A -C889 / allele 1, IL-1A -889T / allele 2, IL-1B +C3953 / allele 1, IL-1B +3953T / allele 2, IL-1RN+T2018 / allele 1, IL-1RN+2018C / allele 2.

5. RESULTS

Surface roughness and substance loss (in vitro – study)

Air polishing alone and with additional rubber-cup polishing using a paste were the only two approaches to cause no enamel loss. Both treatments also entailed less cementum loss (≤ 20 µm) than Ultrasonic Scaling or Hand Instrumentation with and without additional polishing paste, and both yielded the most favorable Roughness values on enamel. Air polishing alone was the only group to reveal no significant change in terms of Roughness from untreated cementum ($p = 0.999$). All other approaches (Ultrasonic, Hand Instrumentation, rubber-cup polishing with abrasive paste) significantly altered the surface roughness of cementum ($p \leq 0.017$) (Arefnia et al., 2020).

a. PREVALENCE OF PERIODONTITIS IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

“A crucial first step in minimizing morbidity and mortality in the aging male population is to identify opportunities for improvement in outcomes of care for the diseases that are most prevalent” (Fenter et al., 2006) Periodontitis is a very high prevalent oral disease, also in highly developed countries (Eke, Paul I. et al., 2018)

From February 2013 until December 2015 414 patients were recruited from the Department of Angiology and sent to the Department of Dental Medicine and Oral Health, giving a referral letter and a precise road map and phone number where to find the corresponding clinic. 5 did not show up without giving reasons, which leaves a screening population of 409 for further analysis. 296 screening patients were male and 113 were female. They were aged between 39 and 89 years with a mean age of 63 years. The mean DMFT was 22,8 and the mean number of natural teeth, including third molars and dental implants was 16,7. The mean grade for the PGU Score was 3,1. PGU 0 was found in 1 proband, PGU 1 in 10 probands, PGU 2 in 115 probands, PGU 3 in 64 probands and PGU 4 in 219 probands (Figure 2). Characteristics of the screening population is shown in Table 7.

Age in years	63 (39-89)
male	296 (72,4%)
female	113 (27,6%)
DMFT	22,8 (5-28)
Number of natural teeth	16,7 (1-31)
PGU 0	1 (0,24%)
PGU 1	10 (2,44%)
PGU 2	115 (28,11%)
PGU 3	64 (15,64%)
PGU 4	219 (53,54%)

Table 7. Characteristics of the Screening Patients (n=409)

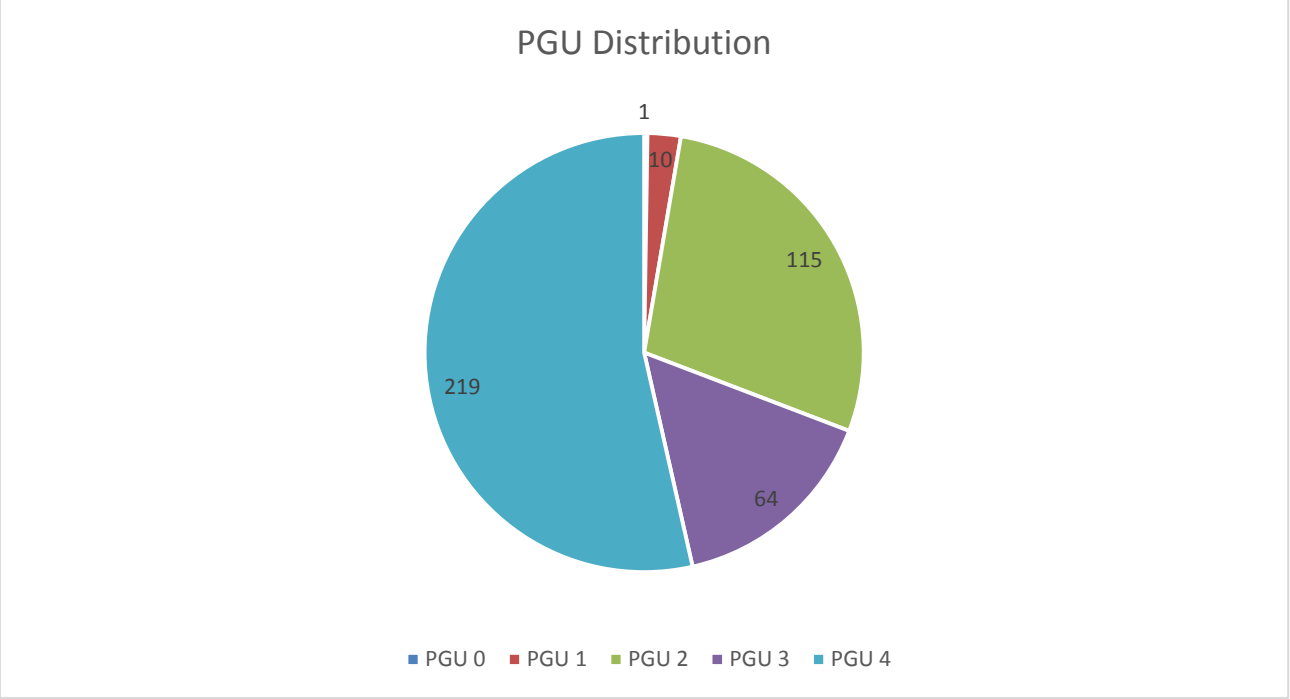

Figure 2. Distribution of PGU Scores of all Screening Patients (numbers of patients/PGU Score)

i. **Adults: 35 – 44 years old**

Nine (38-44 years) of all screening patients were in the adult group. The mean PGU grade was 3.22, the mean number of natural teeth (10-29) was 21.88 and the mean DMFT value (11-28) was 19.00.

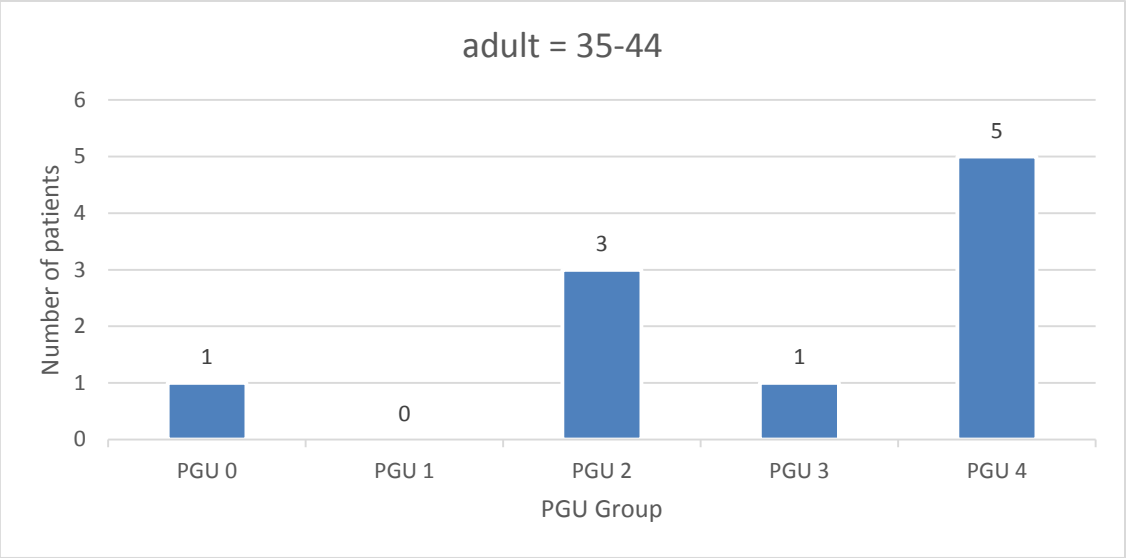

Figure 3. Distribution of PGU Scores (numbers of patients) in the adult screening population (35-44 years)

ii. **Senior Adults: 45 – 64 years old**

210 (45-64 years) of all screening patients were in the senior adult group. The mean PGU grade was 3.31, the mean number of natural teeth (1-32) was 18.87 and the mean DMFT value (13-28) was 21.49.

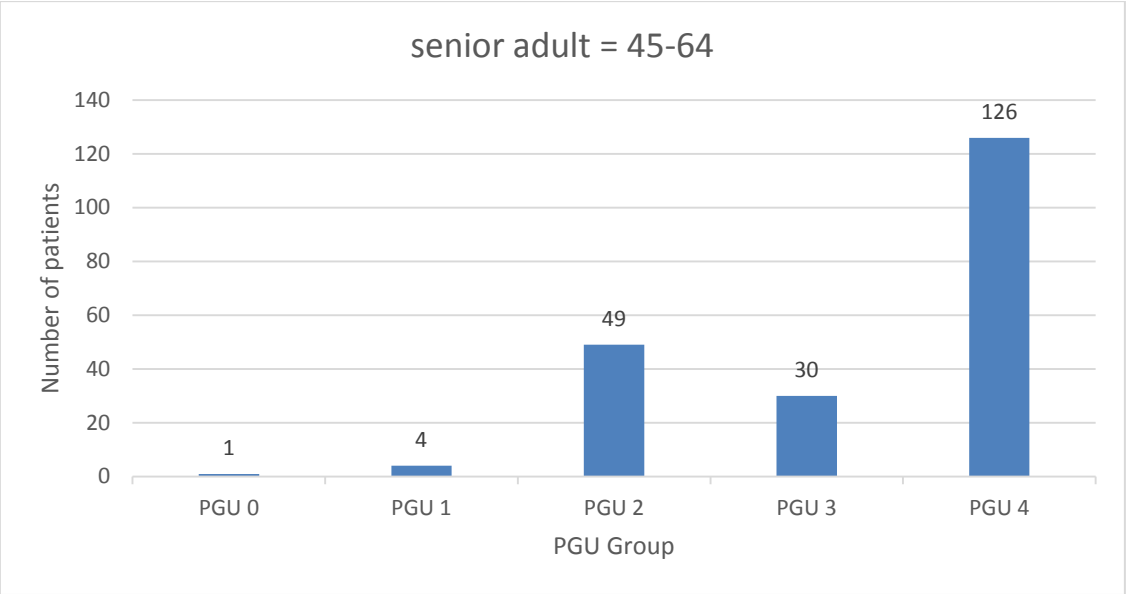

Figure 4. Distribution of PGU Scores (numbers of patients) in the senior adult screening population (45-64 years)

iii. **Young old: 65 – 74 years old**

192 (65-74 years) of all screening patients were in the young old group. The mean PGU grade was 3.07, the mean number of natural teeth (1-29) was 14.19 and the mean DMFT value (10-28) was 24.61.

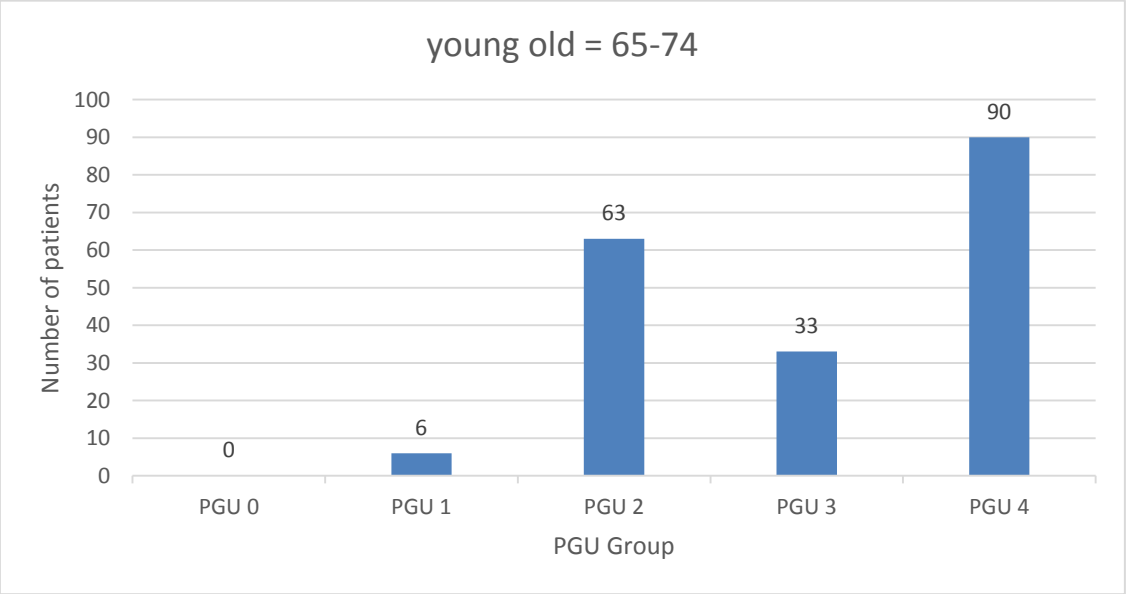

Figure 5. Distribution of PGU Scores (numbers of patients) in the young old screening population (65-74 years)

iv. **Old: 75 – 84 years old**

62 (75-84 years) of all screening patients were in the old group. The mean PGU grade was 2.93, the mean number of natural teeth (1-29) was 13.39 and the mean DMFT value (14-28) was 25.23.

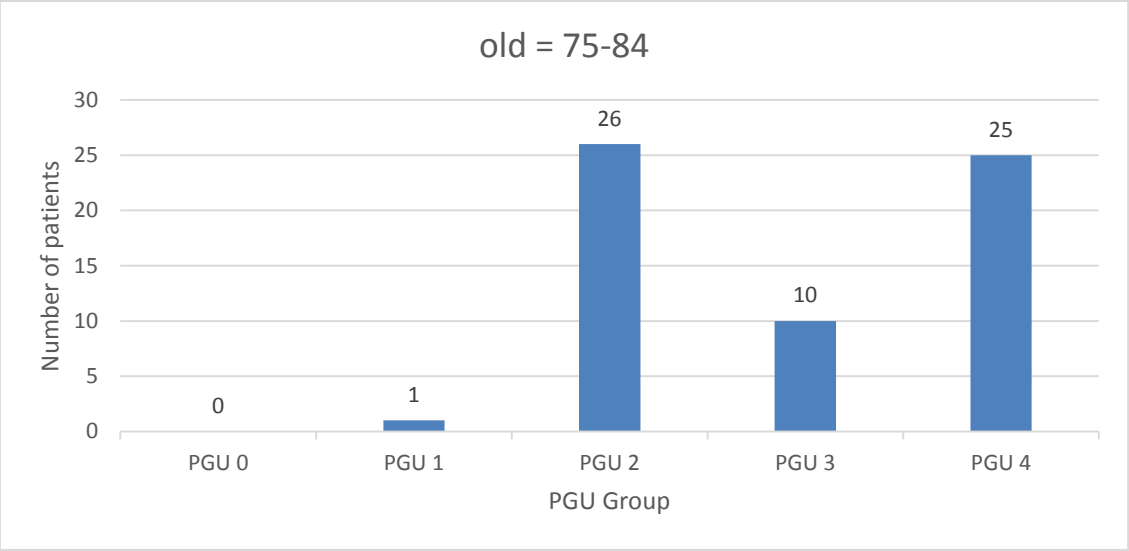

Figure 6. Distribution of PGU Scores (numbers of patients) in the old screening population (75-84 years)

v. **Old-Old: 85 – 100 years old**

6 (85-89 years) of all screening patients were in the old-old group. The mean PGU grade was 3.17, the mean number of natural teeth (6-25) was 13.17 and the mean DMFT value (19-28) was 26.50.

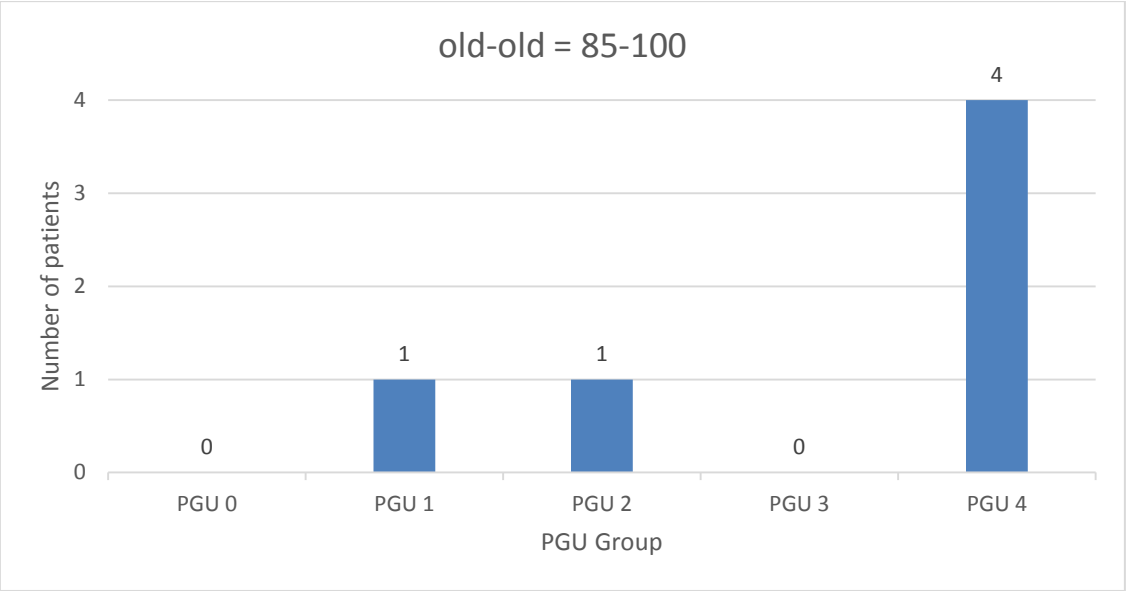

Figure 7. Distribution of PGU Scores (numbers of patients) in the old-old screening population (85—100 years)

vi. **Comparison of the different age groups**

Differences of the age groups of mean PGU, Number of remaining teeth and DMFT are shown in Figure 8 and Figure 9.

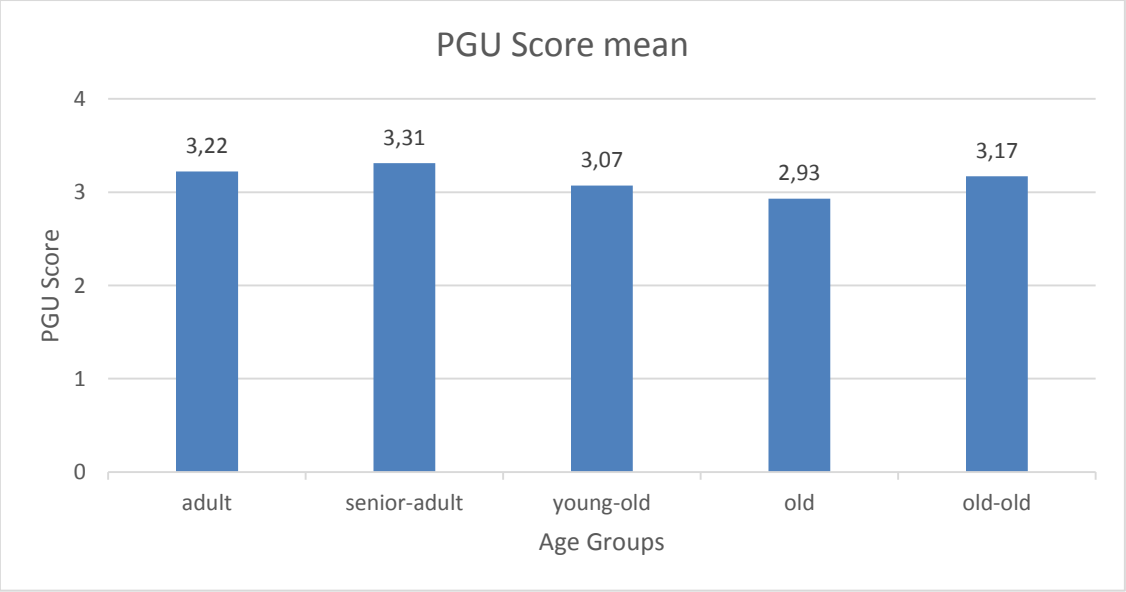

Figure 8. Distribution of PGU Scores in all screening populations

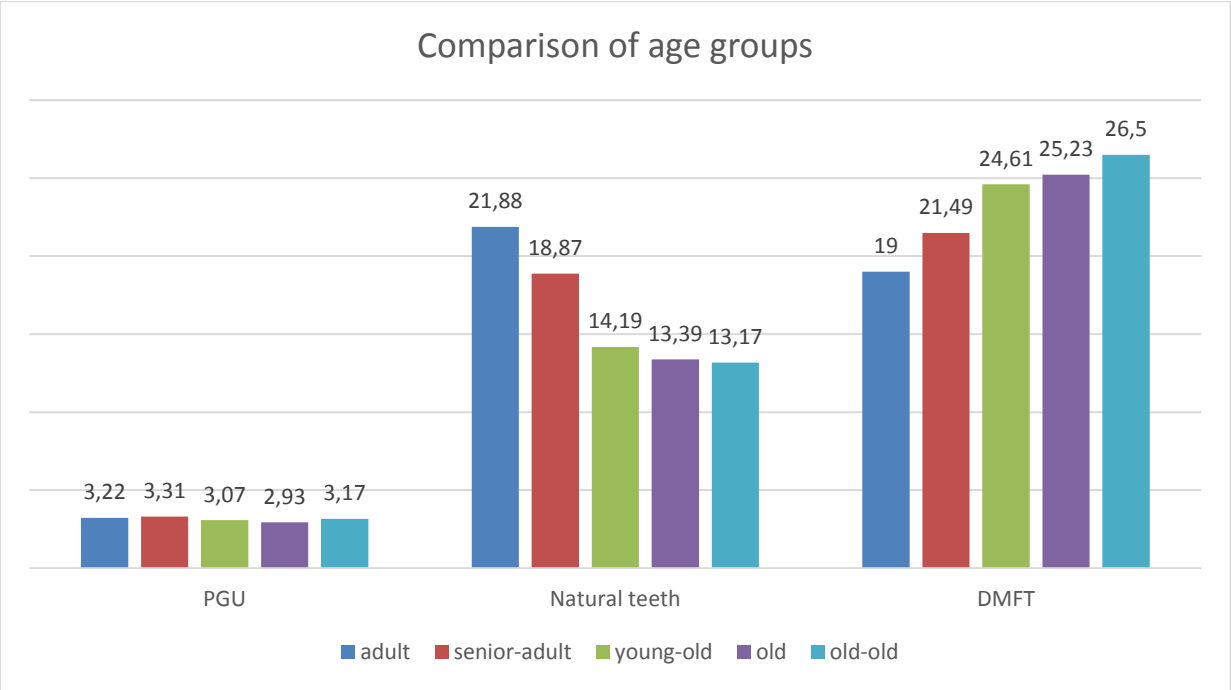

Figure 9. Comparison of mean PGU, number of natural teeth and DMFT of all screening patients in different age groups

vii. **Smoking status among screening patients**

Smoking status among every patient was evaluated at the baseline examination. Smoking habits are recorded in pack/years (PY), a clinical quantification which is calculated by multiplying the pack of cigarettes (20 cigarettes per pack) per day with years of smoking. Of the 409 screening patients 83 were never- or non-smokers (20,29%) and 326 were smokers (Figure 10).

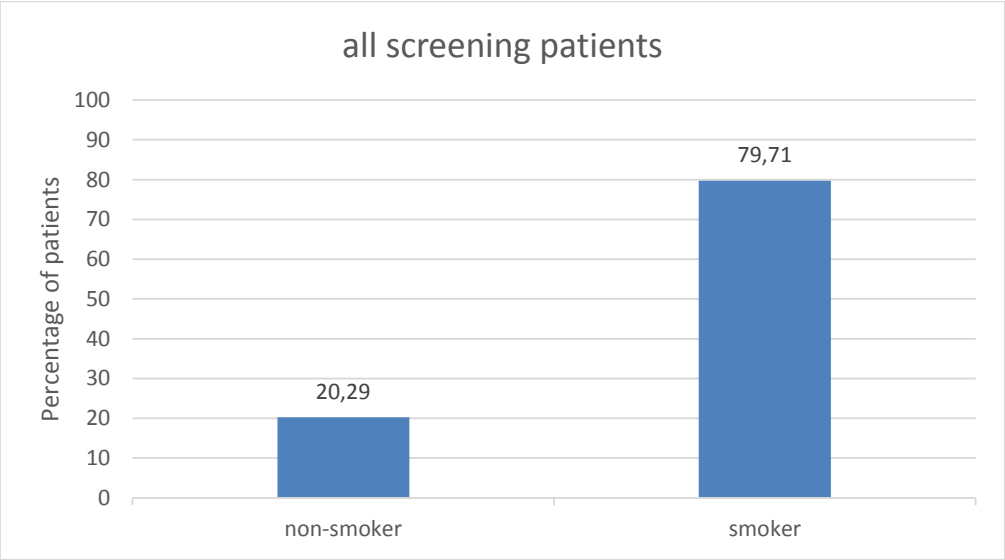

Figure 10. Percentage of non-smokers and smokers in the screening population (n=409)

Of the 296 male patients only 41 were never- or non-smokers (13,85%), 81 had ≤ 30 PY, 125 ≤ 60 PY, 36 ≤ 90 PY and 13 had more than 90 PY (Figure 11).

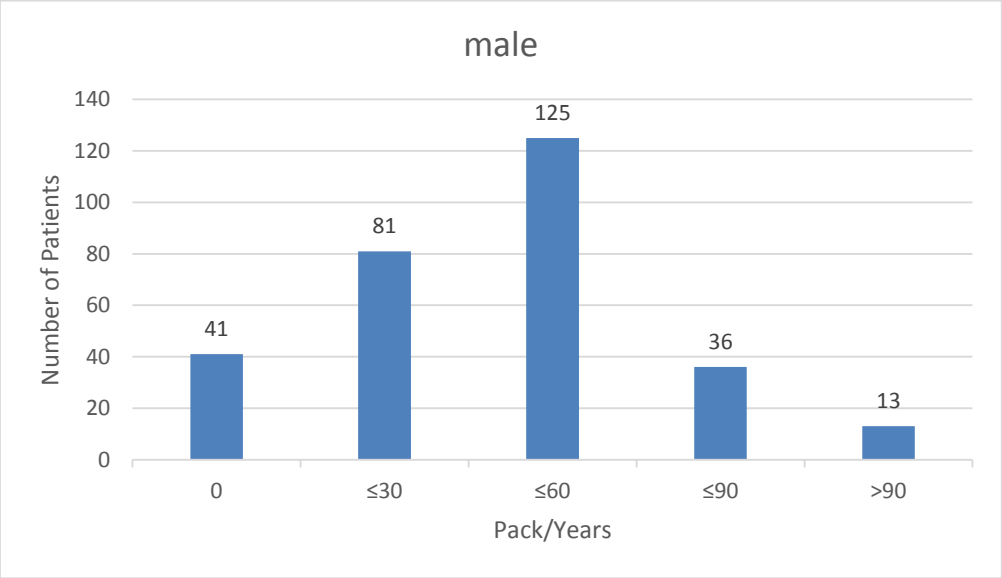

Figure 11. Smoking status in the male screening population (n=296)

Of the 113 female screening patients 42 were never- or non-smokers (37,17%), 39 ≤ 30 PY, 28 ≤ 60 PY, 2 ≤ 90 PY and only 2 had a PY over 90 (Figure 12).

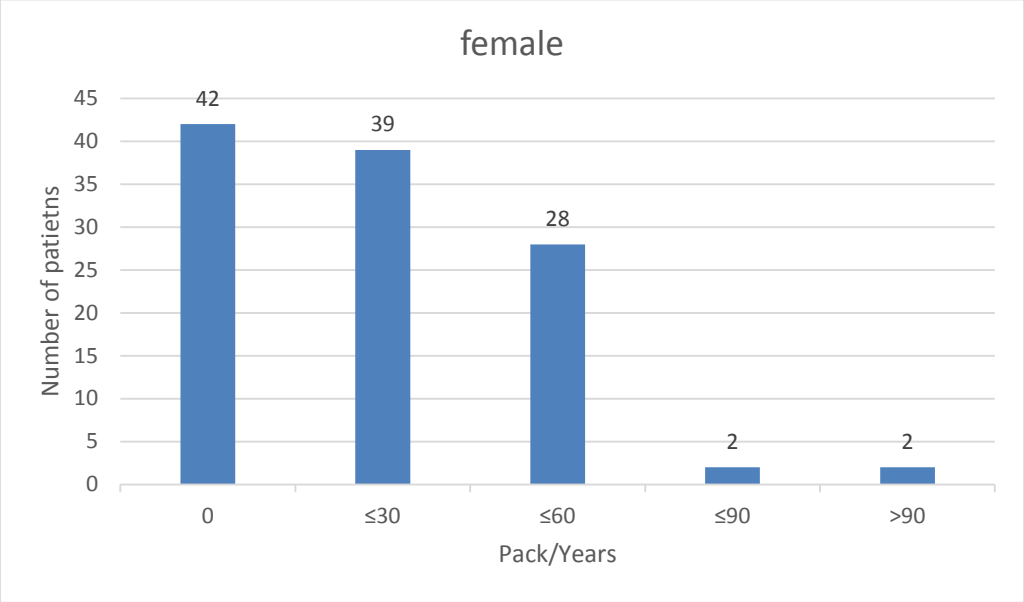

Figure 12. Smoking status in the female screening population (n=113)

b. IMPACT OF PERIODONTAL TREATMENT IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

Non-surgical periodontal treatment is the gold standard therapy form in managing periodontitis and its effectiveness and safety has been shown over decades. On the one hand it is possible to reduce the inflammatory burden by eliminating biofilm and calculus, on the other hand the contact with the patients is quite intense due to several appointments. At that occasions the patients can be monitored and reinstructed in maintaining a proper oral hygiene and motivated to stop periodontal tissue lost and disease progress.

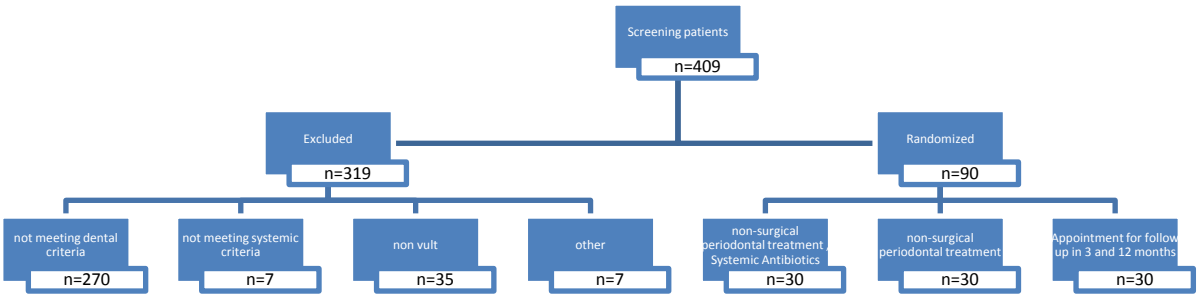

Figure 13. Overview of recruitment process + reasons for excluding screening patients.

i. **Exclusion reasons**

319 patients have been excluded due to dental, systemic or other exclusion criteria. 270 patients did not meet the dental criteria due to:

- Only implant supported bridgework (n=1)
- Not having at least two teeth with probing depth >5mm and/or at least two teeth with clinical attachment loss >5mm (n=134)
- Not having at least twelve natural teeth, including third molars (n=127)
- Not having >20% of sites with bleeding on probing (n=5)
- Periodontal treatment within 6 months of the study (n=3)

Seven patients did not meet the systemic criteria due to:

- Allergy to penicillin (n=4)
- Carcinoma (n=3)

35 patients did not want to participate after explaining the study protocol and seven patients did not participate due to other reasons like:

- Abdominal aneurysm (n=1)
- Participation in another clinical study (n=6)

60 patients were randomized to the PT1 (n=30) and PT2 (n=30) group and invited to receive a non-surgical periodontal treatment. Treatment was performed by a single, experienced dentist, with no time limit and in a closer and highly interactive follow-up. Patients from the PT1 group were advised to take additionally systemic antibiotic (Amoxicillin 500mg + Clavulanic acid 125mg and Metronidazole 500mg) three times daily, every 8 hours, for seven days. If necessary, in both treatment groups the therapy was performed using local anesthesia.

ii. **Control Group**

30 patients were allocated to the CG and received oral hygiene instructions and were given a tentative diagnosis. They were asked not to see any dentist for 3 months except in cases of pain or other acute symptoms. No patient randomized to the CG needed to see a dentist or perform any dental therapy in the study period.

iii. Drop out patients

A total number of five randomized patients dropped out of the study. Four patients (two from PT2 and two from CG) didn't show up for the study appointments after randomization and one patient (PT1) was diagnosed with a lung-carcinoma shorty after randomization and therefore has been excluded. A total of 85 patients attended all study visits in the specified course of time.

iv. Treatment PT1 and PT2

From initial to final treatment, which ended with a full-mouth disinfection (FMD), in both treatment groups it took one to four appointments in a time interval of maximum nine days. Three patients were treated in one session, 42 patients in two sessions, ten in three and two patients received four sessions of non-surgical treatment as shown in Figure 14.

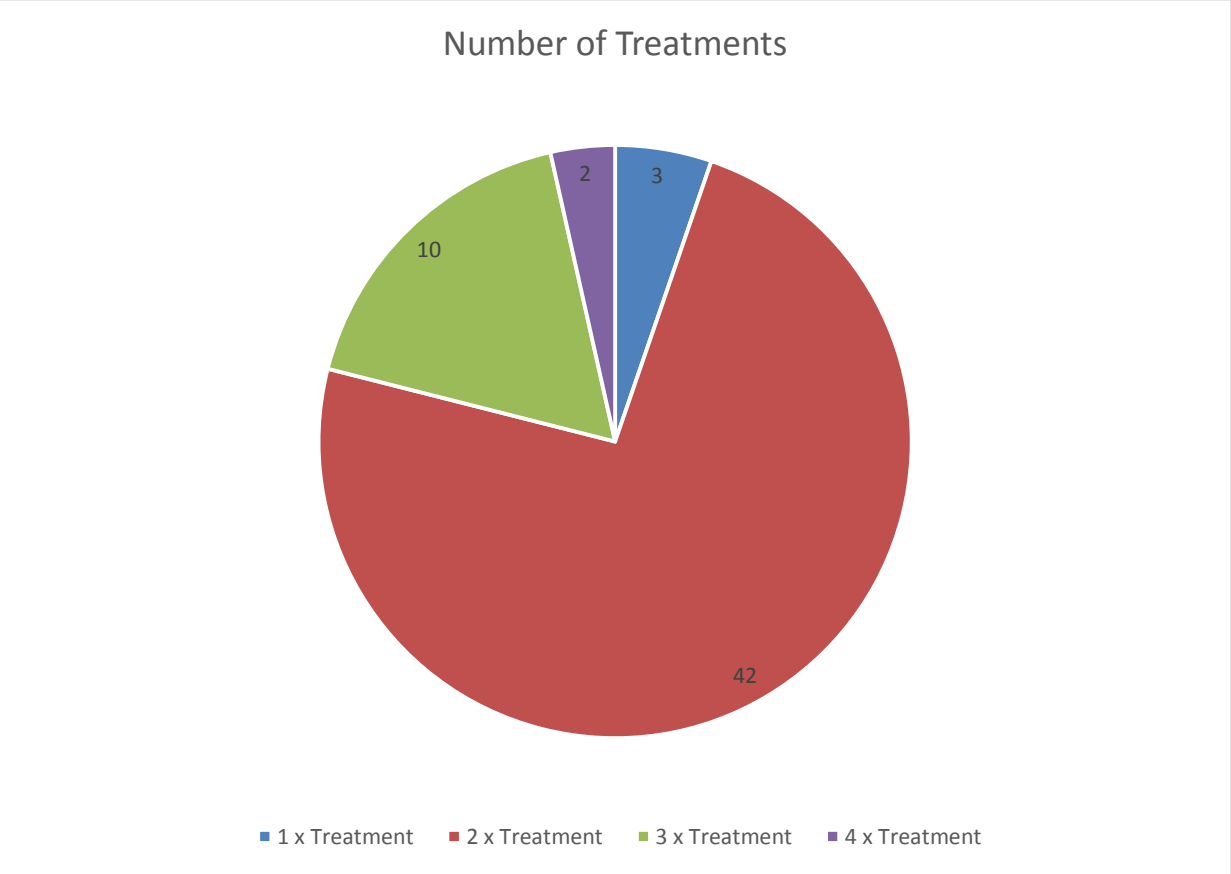

Figure 14. Number of treatment visits in both treatment groups (PT1 and PT2).

All patients from PT1 and PT2 were asked to rinse twice a day with 0,2% of Chlorhexidine rinsing solution (Chlorhexamed forte®, GSK) until the first follow up visit after FMD, which took place in mean after 91,65 days.

v. **Follow up – II**

All study patients, who underwent the first follow-up visit were given an exact periodontal diagnosis and asked to have further treatment done either at their dentists or at the University Clinic for Dental Medicine and Oral Health. They were invited for a second follow-up for another full periodontal examination. From the 85 patients remaining patients from the first follow-up eight did not show up for the second follow-up visit. All of them, two from PT1, three from PT2 and three from CG, without giving any specific reason. The remaining 77 patients received a full periodontal status, using the Florida-Probe® System, by the same blinded, calibrated examiner who did the initial status and follow-up I.

vi. **PISA and reduction of inflammation**

The PISA values were calculated using the excel sheet from the initial authors of the PISA index (Nesse et al., 2008). A Microsoft Excel developer tool was used to create a macro, which can automatically calculate the PISA value from the Florida-Probe® Excel sheets and the differences of the PISA values at the three different time points.

1. Statistics

The three groups and three time points were statistically evaluated with a general linear model with repeated measurement. For reassurance, that all three study groups are comparable at the baseline inflammation level the three groups were compared with a one-factor variance analysis. There is no initial significant difference in the PISA value at the baseline examination time point ($p = 0,395$).

2. PT1

The mean baseline value for PISA in the PT1 group was 842, for follow-up I it was 263 and for follow-up II it was 293.

3. PT2

The mean baseline value for PISA in the PT2 group was 765, for follow-up I it was 469 and for follow-up II it was 441.

4. CG

The mean baseline value for PISA in the CG group was 919, for follow-up I it was 818 and for follow-up II it was 643.

5. Comparison of study groups: PISA

There was a significant change in all three groups over time ($p < 0.001$) shown in Figure 15. The change over time is significantly different between the groups ($p < 0.001$). The PT1 group sinks faster than the PT2 and CG groups. In addition, the PISA values at follow-up were significantly different ($p = 0.002$).

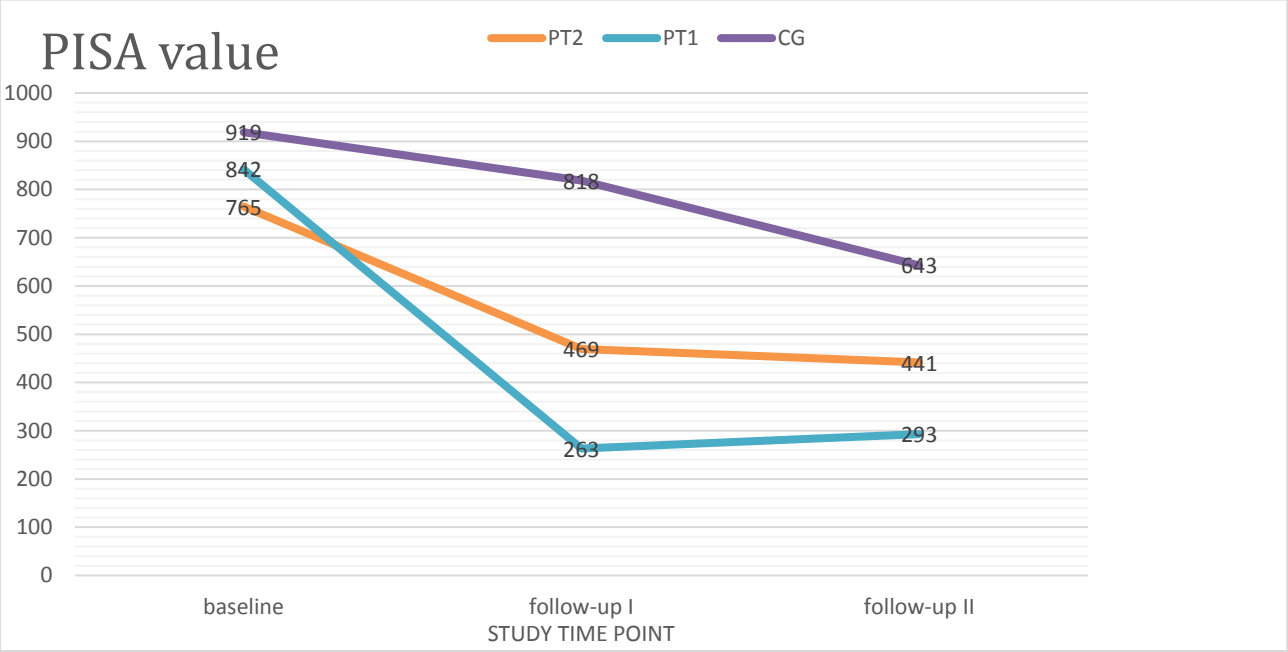

Figure 15. PISA values in study group comparison for every study time point.

vii. **Molecular/microbial diagnostics**

The data were evaluated descriptively and exploratively with a significance level of 5%. Since the main target size is ordinal scaled, nonparametric tests were used. The nonparametric Kruskal-Wallis test showed no significant difference between the baseline values of the three groups for any of the investigated bacteria species.

Partial results of the semiquantitative assessment of eleven suspected periodonto-pathogenic bacteria (Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia and Treponema denticola, Parvimonas micra, Fusobacterium nucleatum, Campylobacter rectus, Eubacterium nodatum, Eikenella corrodens and Capnocytophaga sp.) have been subject for a diploma thesis, submitted at the Medical University of Graz (“Microbial diagnostics in periodontal therapy. Investigations within a prospective clinical study.” by DDr. Stefanie Mohadjer).

Representative results from baseline to follow-up I for the efficacy of adjunctive systemic antibiotics in non-surgical periodontal treatment are shown in Table 8. Table 9 shows the changes between Follow-up II and baseline assessment and Table 10 from Follow-up II to Follow-up I.

Bacteria	PT1	PT2	CG
Aggregatibacter a.	p = 0,202	p = 0,655	p = 0,414
Porphyromonas g.	p = 0,003	p = 0,205	p = 0,705
Prevotella i.	p = 0,244	p = 0,756	p = 0,287
Tannerella f.	p = 0,001	p = 0,202	p = 0,564
Treponema d.	p = 0,000	p = 0,399	p = 0,776
Parvimonas m.	p = 0,768	p = 0,833	p = 0,377
Fusobacterium n.	p = 0,739	p = 0,366	p = 0,336
Campylobacter r.	p = 0,003	p = 0,297	p = 0,040
Eubacterium n.	p = 0,001	p = 0,177	p = 0,661
Eikenella c.	p = 0,099	p = 0,060	p = 0,937
Capnocytophaga sp.	p = 0,009	p = 0,501	p = 0,252

Table 8. Changes between Follow-up I and Baseline assessment of microbiological data.

Bacteria	PT1	PT2	CG
Aggregatibacter a.	p = 0,131	p = 0,999	p = 0,655
Porphyromonas g.	p = 0,092	p = 0,461	p = 0,564
Prevotella i.	p = 0,026	p = 0,557	p = 0,498
Tannerella f.	p = 0,010	p = 0,398	p = 0,564
Treponema d.	p = 0,001	p = 0,785	p = 0,717
Parvimonas m.	p = 0,686	p = 0,489	p = 0,750
Fusobacterium n.	p = 0,527	p = 0,705	p = 0,317
Campylobacter r.	p = 0,014	p = 0,630	p = 0,421
Eubacterium n.	p = 0,028	p = 0,842	p = 0,724
Eikenella c.	p = 0,678	p = 0,183	p = 0,796
Capnocytophaga sp.	p = 0,019	p = 0,158	p = 0,348

Table 9. Changes between Follow-up II and Baseline assessment of microbiological data.

Bacteria	PT1	PT2	CG
Aggregatibacter a.	p = 0,317	p = 0,317	p = 0,655
Porphyromonas g.	p = 0,011	p = 0,357	p = 0,480
Prevotella i.	p = 0,999	p = 0,469	p = 0,213
Tannerella f.	p = 0,006	p = 0,276	p = 0,999
Treponema d.	p = 0,061	p = 0,251	p = 0,676
Parvimonas m.	p = 0,700	p = 0,370	p = 0,982
Fusobacterium n.	p = 0,564	p = 0,317	p = 0,999
Campylobacter r.	p = 0,089	p = 0,810	p = 0,098
Eubacterium n.	p = 0,017	p = 0,225	p = 0,850
Eikenella c.	p = 0,210	p = 0,859	p = 0,457
Capnocytophaga sp.	p = 0,389	p = 0,144	p = 0,823

Table 10. Changes between Follow-up II and Follow-up I assessment of microbiological data.

In every time point comparison almost only in the antibiotic group there was a significant change in the bacterial load of the investigated species. Significance was set at $p < 0.05$. The biggest changes are observed during the follow-up I and baseline and between follow-up II and baseline time points.

viii. **MMP-8**

To compare the three study groups, a single factor variance analysis was performed. Once for the difference between baseline and follow-up I examination and once also for follow-up I and follow-up II. Significant level was set at $p < 0.05$. From baseline to follow-up I examination a significant statistical difference can be shown ($p = 0.003$). The CG is significantly different from PT2 and even more from PT1. On the other hand, there is no statistical significance between the PT2 and PT1 groups on the level of αMMP-8. From the timepoints follow-up I and follow-up II there could be shown, that CG differs significantly from PT1 ($p = 0.008$). No difference occurred between CG and PT 2. Changes and values are highlighted in Figure 16 and Table 11.

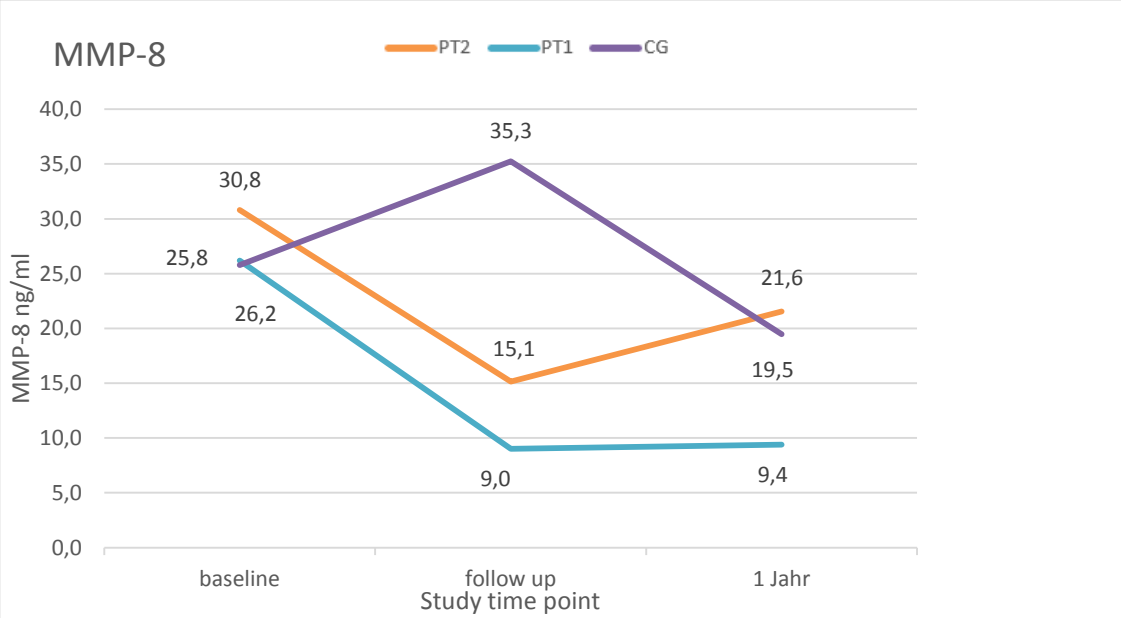

Figure 16. αMMP-8 levels (ng/ml) group comparison for every study time point.

Group	Difference	Minimum	Maximum	Mean	SD
PT2	follow-up I – baseline	-65	41	-15,68	25,84
	follow-up II – follow-up I	-21	49	7,20	17,48
PT 1	follow-up I - baseline	-130	12	-17,68	28,50
	follow-up II – follow-up I	-17	19	0,67	8,41
CG	follow-up I - baseline	-38	130	9,48	38,09
	follow-up II – follow-up I	-167	41	-15,46	40,24

Table 11. Differences, minimum, maximum, mean-values and standard deviation (SD) of αMMP-8 values (ng/ml) at the investigated time points for every study group.

ix. **Interleukin-1 Gene-polymorphism**

Excluding the drop-out patients, 85 patients underwent the test for IL-1 Genotype (PST). Overall 26 were Genotype negative (Genotype 1) and 59 were positive. 19 were Genotype 2, 15 were Genotype 3 and 25 were Genotype 4. Figure 17 to 20 show overall and group specific distribution of IL-1 Genotype in this study population.

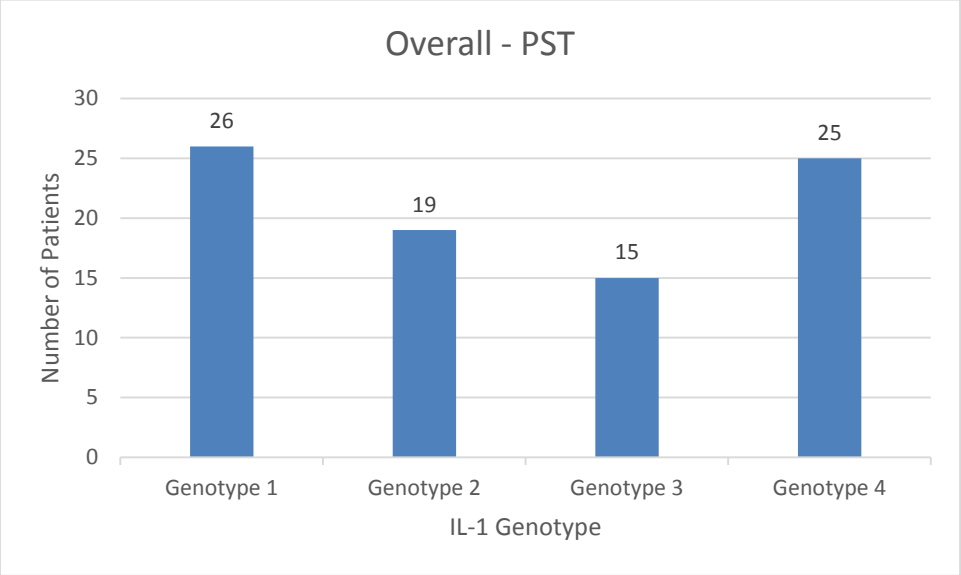

Figure 17. Distribution of IL-1 Genotype groups in the whole study population.

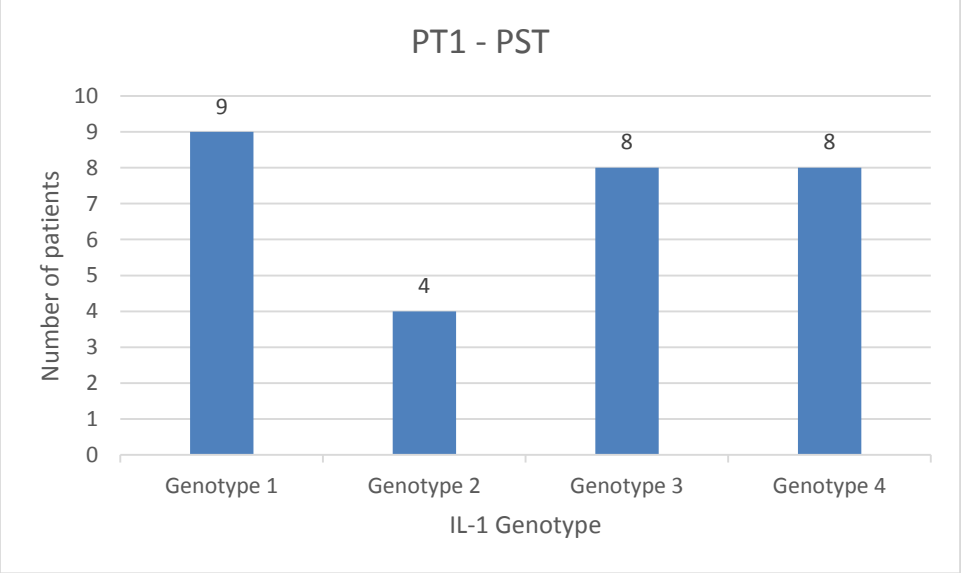

Figure 18. Distribution of IL-1 Genotype groups in the PT1 study population.

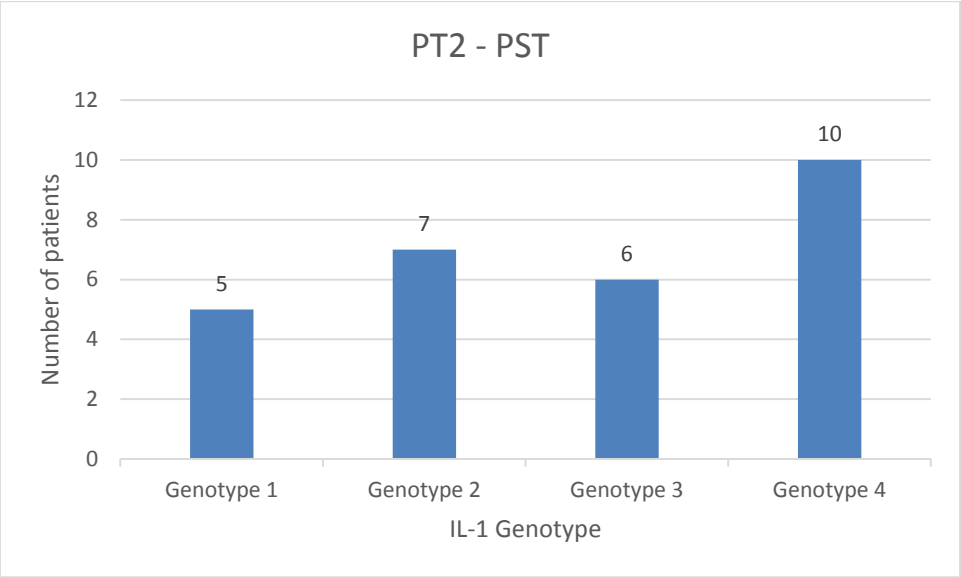

Figure 19. Distribution of IL-1 Genotype groups in the PT2 study population.

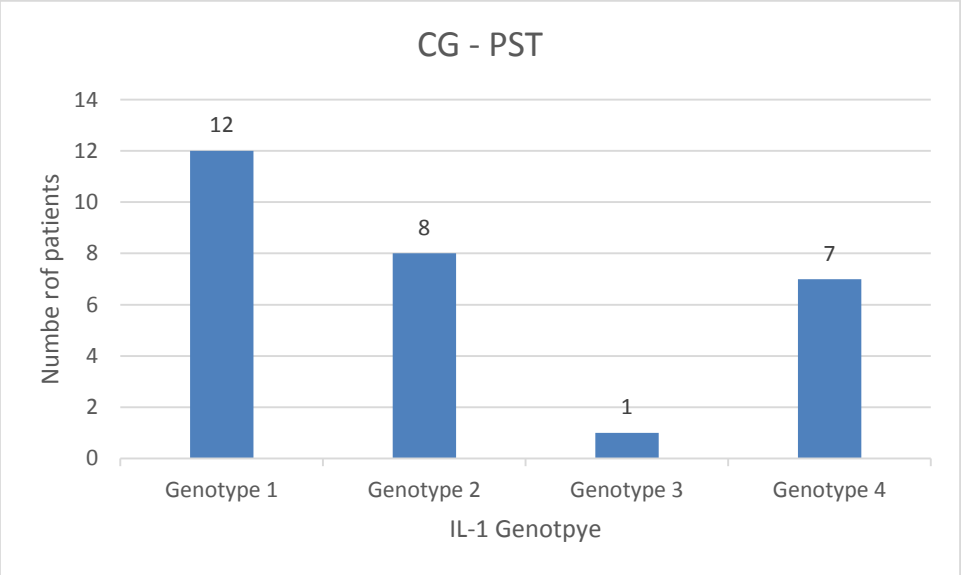

Figure 20. Distribution of IL-1 Genotype groups in the CG study population.

6. DISCUSSION

This dissertation intended mainly to highlight two different scientific questions: how high is the prevalence of periodontal diseases in a cohort of patients with PAD, and what is the impact of different non-surgical periodontal treatment methods on a clinical and microbiological level compared to a control group without any intervention? To fulfill the answer to these topics, patients from a joint clinical project were invited to participate. 409 patients were successfully be screened and 90 were included and randomized for further study procedures.

Approximately ten years ago, the Periodontitis and Vascular Events (PAVE) Study(Offenbacher, Steven et al., 2009) results were published. 282 subjects were analyzed in this multicenter clinical trial which lasted for 2.5 years. The authors of this landmark study concluded, that *"periodontal therapy may lower hs-CRP levels among non-obese cardiovascular patients if the levels are >3 mg/l initially, and it may prevent a drift to levels >3 mg/l for those that are in the intermediate range of 1 to 3 mg/l. It does not appear that periodontal therapy diminishes hs-CRP levels among individuals with hs-CRP levels <3 mg/l in this patient population."*. The authors stated that significant improvements in periodontal health could be achieved when a periodontal therapy took place compared to a no-therapy cohort. Even if the improvement on periodontal parameters is not surprising at all, the implications of this trial are far-reaching. It could be demonstrated, that with adjusted periodontal parameters and higher sample size interventional trials in this specific cardiovascular group of patients is feasible. On this basis this joint clinical project was designed and funded by the FWF.

For a better readability the discussion of this dissertation is also divided in two parts.

Additionally, the results of our in – vitro study for surface roughness are consistent with the literature (Bühler et al., 2015). Even if a current study with fifty incisor and premolar roots showed that hand instruments yielded in smoother surfaces than ultrasonic scalers (Poornima, Meena, & Pratibha, 2019) comparison is questionable because in our study only third molars have been used. Air – polishing technology with non – abrasive powders based on Erythritol and Chlorhexidine caused no substance loss of enamel and —a s the only technique evaluated — even no substance loss of cementum or surface alterations.

a. Part I: PREVALENCE OF PERIODONTITIS IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

It is quite astonishing that even if periodontitis is considered as one of the most prevalent chronic non-communicable diseases worldwide (Demmer & Papapanou, 2010) the periodontal status of populations globally of the 21st century is not known (Dye, 2012). The WHO introduced a global oral health database where also periodontal conditions are examined using the Community Periodontal Index (CPI). The objective is to present information on dental health or disease for a variety of participating countries. The electronic data servers for periodontal conditions are located in Japan at the Niigata University and the information is being constantly updated. In epidemiological studies, estimations of around 20-30% of the adult population are suffering from moderate to advanced periodontitis (Holtfreter, Schwahn, Biffar, & Kocher, 2009; Norderyd & Hugoson, 1998). Two approaches for standardized periodontal disease definitions (Page & Eke, 2007; Tonetti, MS, Claffey, & European Workshop in Periodontology group C, 2005) led to an increased number of epidemiological studies and more-or-less comparable results. In 2015 the Joint EU/USA Periodontal Epidemiology Working Group published proposed standards for reporting chronic periodontitis prevalence and severity in epidemiologic studies (Holtfreter et al., 2015). These standards should enhance the comparisons between epidemiological studies and overcome potential pitfalls like different measurement techniques or case definitions.

In the USA a National Health and Nutrition Examination Survey (NHANES) data analysis of 3585 participants over 40 years of age and an ABI of < 0.90 was evaluated for the years 1999 to 2002. After adjusting for age, gender, race, poverty, traditional risk factors of PAD, periodontitis was significantly associated with PAD (Lu, Parker, & Eaton, 2008).

Another large cross-sectional study with 1343 adults aged over 40 years were screened for the association of periodontitis and the development of early atherosclerotic vascular disease in Korean adults (Ahn et al., 2016). PAD was defined when ABI was ≤ 1 and diagnosis of periodontitis was assessed by RBL. Severe periodontitis (≥2 non-adjacent interproximal sites with RBL ≥ 6mm) was associated with PAD (adjusted OR = 2.03; 95% CI: 1.05–3.93).

A research group from the San Luis Potosí University, Mexico showed, that PAD is associated with a higher risk for periodontitis than in a control healthy group(Soto-Barreras et al., 2013) . 30 patients >40 years of age with PAD, diagnosed by an ABI value of ≤ 0.90

in either leg with concomitant periodontitis, diagnosis determined when AL was ≥ 4mm were compared to a group of 30 patients without PAD (ABI > 0.90).

Like in our study groups, subgingival biofilm was collected and analysed with PCR assay. In contrary to our study, the analysis contained only five periodontopathogens (P.gingivalis, T.forsythia, P. intermedia, T. denticola and A. actionmycetemcomitans) but also one key stone bacteria for dental caries (S. mutans). Furthermore, DMFT index was also evaluated. In fact, it was the first evaluation of the DMFT in a cohort of PAD patients ever. The DMFT index was significantly higher ($p = 0.0002$) in patients with PAD (21.4 ± 2.6) than in no-PAD patients (18.3 ± 3.4). Especially tooth loss was higher in the PAD group, which is consistent with other cohort studies (Hung et al., 2003). Subgingival biofilm composition did not differ in both groups and the risk for having PAD was six-fold increased when over 30% of probing sites had CAL ≥ 4mm.

Another research group from the Erciyes University, Turkey performed an evaluation of the relationship between PAD and periodontal disease by examining the levels of inflammatory cytokines, namely pentraxin 3 (PTX-3) and IL-1 β and high sensitive C-reactive protein (hs-CRP) (Çalapkorur, Alkan, Tasdemir, Akcali, & Saatci, 2017). Patients with an ABI value of ≤ 0.90 were diagnosed as PAD and an ABI of > 1.00 was diagnosed as health control. 40 PAD and 20 non-PAD patients were invited to the study. DMFT index was also evaluated and was also lower in the non-PAD group (12.65 ± 6.30) than in the PAD group (14.60 ± 5.46) but not significantly ($p = 0.221$). The mean age ± SD was 57.4 ± 11 years and 60.4 ± 9 years for the non-PAD and PAD group, respectively which is quite similar like in our PAD population. There were significantly ($p < 0.05$) more patients with gingivitis in the non-PAD group, most patients in the PAD group had localized and generalized chronic periodontitis. The number of sites with PD ≥ 5 mm was significantly higher in the PAD group as compared with the non-PAD group ($p = 0.019$). Inflammatory cytokine (IL-1β, PTX-3) and acute phase protein (hs-CRP) levels from both gingival crevicular fluid and serum were not different between the two groups ($p > 0.05$).

To date, there is no comprehensive epidemiological study about the dental or periodontal status of the general Austrian population. To compare the screening population to a representative cohort all collected dental data is discussed in comparison with the Fifth German Oral Health Study (DMS V)(Jordan et al., 2014). The study cohort are compared separated by age using following groups: 35-44, 65-74 and 75-100 years.

i. **35 – 44**

In the DMS V the mean DMFT index for male and female in the group of 35-44 was 12.4 compared to 19 in the screening population. The CPI, which is comparable to the PGU was 41.3% for Grades 0,1 and 2, 48,3% for Grade 3 and 10.4% Grade 4 in the DMS V compared to 40%, 10% and 50% respectively in the study population. The mean number of missing teeth including wisdom teeth in the DMS V was 4.8 compared to 10.1 in the screening population.

ii. **65 – 74**

In the DMS V the mean DMFT index for male and female in the group of 65-74 was 17.7 compared to 24.2 in the screening population. The CPI, which is comparable to the PGU was 24.6% for Grades 0,1 and 2, 50.8% for Grade 3 and 24.6% Grade 4 in the DMS V compared to 32.25%, 18.54% and 49.19% respectively in the study population. The mean number of missing teeth including wisdom teeth in the DMS V was 14.6 compared to 17.3 in the screening population.

iii. **75 – 100**

In the DMS V the mean DMFT index for male and female in the group of 75-100 was 21.6 compared to 25.3 in the screening population. The CPI, which is comparable to the PGU was 19.4% for Grades 0,1 and 2, 50.5% for Grade 3 and 30.1% Grade 4 in the DMS V compared to 42.64%, 14.71% and 42.65% respectively in the study population. The mean number of missing teeth including wisdom teeth in the DMS V was 21.6 compared to 18.6 in the screening population.

iv. **Cigarette smoking**

To better understand a possible link or influences between periodontal disease and systemic conditions one has to be aware, that one major risk factor for both -cigarette smoking – is also a major confounder in epidemiological studies. One of the most insightful reviews recently explains the fundamentals of periodontitis - systemic disease associations in the presence of smoking (Hujoel, Drangsholt, Spiekerman, & DeRouen, 2002). The authors clearly describe the four lines of evidence which suggest that the by the time observed periodontitis-systemic disease associations were in part a result of confounding by smoking:

1. *no periodontitis–systemic disease associations have been identified among never-smokers*
2. *periodontitis and smoking are associated with similar health risks*
3. *conflicting study results can be explained in terms of statistical adjustment for tobacco smoking*
4. *dental infection elimination through complete tooth removal, unlike smoking cessation, does not reduce health risks.*

The authors concluded that studies reporting a periodontitis-systemic disease association should be done among healthy never-smokers.

Since smoking cigarettes is the most aggravating behavioural factor for periodontal disease comparison with the general Austrian population makes sense to better comprehend the reduced and strongly affected periodontal health.

23.9% of the population of Austria were daily smokers of cigarettes in the year 2014 according to the statistical institute of the European Union. The average percentage of smokers in the 28 countries of the European Union was 18.4% at the same time. In the screening population overall 79.4% were smokers, most of them heavy smokers. In the male cohort 86.15% and in the female cohort 62.83% were smokers. This high prevalence of smokers in the screening population is alarming but not very astonishing since smokers have a 2.3 fold higher prevalence for PAD and in countries like Austria where approximately 30% of the population are smokers, 50% of PAD can be attributed to smoking (Willigendael et al., 2004). Successful smoking cessation programs can considerably be part of a more favourable periodontal health or prevention of disease (Tomar & Asma, 2000) and may reduce a common potential risk factor for different diseases.

v. Summary

Prevalence of periodontal disease compared to a representative cohort in a European neighbour country (Germany) was significantly higher in the screening population, especially the severe cases. The higher prevalence of smoking, DMFT index and almost in every study population higher number of missing teeth underscores the statement that the dental status of this screening population of patients with PAD was inferior to the healthy general public. This could be also due to the fact that both diseases share common underlining risk factors.

IMPACT OF PERIODONTAL TREATMENT IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

Non-surgical periodontal treatment with subgingival debridement is an effective treatment and reduces inflammation, in terms of lower BOP, reduced PD and CAL gain in patients with periodontitis (Cobb, 2002; Van der Weijden & Timmerman, 2002a). Oral hygiene instructions and supragingival plaque control are also key elements in improvement of periodontal health. Successful therapy is also dependent on tooth type, furcation involvement, severity of periodontal disease at intake and smoking status (Van der Weijden, Dekkers, & Slot, 2019). The positive and successful effects of non-surgical periodontal treatment are published since the early 80's of the last century. In clinical studies the use of hand or ultrasonic instruments was pushed to it's not known potential (Badersten, Nilvéus, & Egelberg, 1981; Badersten, Nilveus, & Egelberg, 1984) and the need of surgical interventions decreased rapidly. Since non-surgical treatment also has limits (Claffey, Loos, Gantes, Martin, & Egelberg, 1989) (Goodson et al., 2012) adjunctive therapies were introduced to eradicate the bacterial infection with the use of antibiotics (Baer & Socransky, 1979). Great importance was attached to the fact, that not all bacterial species in the mouth are pathogens but host compatible or beneficial (Socransky & Haffajee, 1997). Particular emphasis was laid on eradication of more specific species and an approach to target key stone periodontopathogenic bacteria like Aggregatibacter actinomycetemcomitans or Porphyromonas gingivalis was established (Van Winkelhoff et al., 1989a) not only for patients with, formerly known as juvenile or aggressive periodontitis (Lindhe & Liljenberg, 1984), but also for adult periodontitis (Winkel, Van Winkelhoff, Timmerman, Van der Velden, & Van der Weijden, 2001) (Grellmann, Sfreddo, Maier, Lenzi, & Zanatta, 2016) and periodontitis with systemic diseases. Thus, the combination of β-lactam antibiotics, amoxicillin and nitroimidazole, metronidazole is the most used adjuvant antibiotic medication in periodontal treatment protocols. However, it showed contradictory results since its introduction (Van Winkelhoff et al., 1989b)Eradication effects could be observed (Aimetti, Romano, Guzzi, & Carnevale, 2012; Mestnik et al., 2012) as well as no-effects on the targeted pathogens (Heller et al., 2011; Xajigeorgiou, Sakellari, Slini, Baka, & Konstantinidis, 2006). On a clinical level a variety of interventional trials with antibiotics used changes in PD or CAL as the primary outcome parameter. Since in most studies the initial PD was determined with ≥ 7 mm, the clinical relevance was questioned since pockets with 8 mm or 7 mm both need additional treatment in most cases (Feres et al., 2012; Sampaio et al., 2011). But even if the inhibition of key stone periodontopathogens is achievable with

adjuvant systemic antibiotic therapy and an improvement of biofilm composition is more likely (Hajishengallis & Lamont, 2012) one big issue remains: antibiotic resistance.
The German Society of Periodontology (DGP) published very recently S3-guidelines for the use of antibiotics in periodontal treatment (Hezel, 2019) . This very comprehensive guidelines tried to give clear recommendations on timepoint, indication of use for chronic and aggressive periodontitis, concomitant diabetes or smoking, which class of antibiotics and which dosage should be used. Chronic periodontitis patients of 56 years or older should primarily not get systemic antibiotics. Since chronic periodontitis is very common in this age group the adjuvant antibiotic therapy would be narrowed down a lot.

In our particular research project, out of 409 screening patients 90 participated in this prospective interventional three-armed trial. After inclusion participants were randomized allocated to one of three study groups. Periodontal therapy (PT) consisted of non-surgical therapy and debridement of supra- and subgingival biofilm or calculus with or without use of systemic antibiotics. One single experienced periodontist did all the procedures and gave precise instructions for oral hygiene procedures for the patient's home care. Chlorhexidine was used in different concentrations chairside (0,2% - 1%) and for home use (0,2% twice a day) according to well-known protocols and seems to be efficient when used as subgingival irrigation (Prietto et al., 2020). Since bacteria of oral origin enter the circulation following physical perturbations, especially after dental procedures like tooth polishing, scaling, tooth extraction, surgical extraction of third molar and periodontal probing(Reyes, Herrera, Kozarov, Roldán, & Progulske-Fox, 2013b), we wanted to minimize this risk with the additional use of chlorhexidine in higher concentrations.
Participants of the CG were given a tentative diagnosis and were asked not to see any dentist during the first study interval for 3 months. Since no therapy was conducted no benefit on a clinical level could be observed. After follow – up I patients from the CG were asked to see a dentist for further periodontal treatment. Interestingly no major effects in terms of reduction of inflammation (PISA) could be observed.
The PT1 group received systemic antibiotics, amoxicillin 500mg + clavulanic acid 125mg and metronidazole 500mg for seven days, three times a day. Despite the fact of clear evidence for adjuvant systemic antibiotics in subgingival debridement we followed the mentioned protocol. As this procedure is well documented in the literature, alternative models with lower dosage and shorter or longer period of taking were refused during the genesis of the study protocol because it could additionally lower the compliance of patients (Greenberg, 1984). Furthermore, antibiotic resistance problems are not so serious

for this study population. No adverse reaction to any of the performed treatments or used medication or antimicrobial agents occurred during the whole study time.
To quantify and compare the inflammatory burden in the periodontal tissue at the different time points PISA scores was calculated. This scoring system is also the suggested methodology for selected populations in interventional studies for linking periodontal disease and CVD(Tonetti & Van Dyke, 2013). Since no initial significant (p=0.395) different PISA values were found, group comparison was possible.
No change could be observed in the control group, however both interventional groups led to a significant reduction of periodontal inflammation (FIG?). This was expectable (Van der Weijden & Timmerman, 2002b) as non-surgical periodontal treatment is effective in general healthy patients and also in patients with systemic diseases.
We could show that the adjunct use of systemic antibiotics, namely amoxicillin plus clavulanic – acid and metronidazole, was significantly better in terms of reduction of PISA ($p < 0.001$) than non-surgical therapy alone in this study group. FIG?Consistent with the guidelines of the DGP the antibiotic treatment started simultaneously with the subgingival debridement.
The link of periodontitis and systemic diseases created the scientific field of “periodontal medicine”. Since the biological evidence for a plausible cause-effect relationship and influence for a limited number of systemic diseases - like type 2 diabetes - (Taylor, 2001) is given, interventional trials with the goal to show a beneficial effect on the general health via periodontal treatment were conducted in a growing number. Type 2 diabetes is a huge public health issue and doubles or triples the risk for developing periodontitis (Taylor et al., 1996). Periodontitis adversely influences glycemic control in type 2 diabetes patients (Sammalkorpi, 1989) and evidence, that periodontal treatment influences elevated HbA1c values has been gathered also in systematic reviews(Engebretson & Kocher, 2013; Simpson, Needleman, Wild, Moles, & Mills, 2010; Teeuw, Gerdes, & Loos, 2010).
Patients suffering from periodontitis have a 14-15% higher risk for developing CVD (Bahekar, Singh, Saha, Molnar, & Arora, 2007). Evidence that periodontal treatment has a beneficial effect on CVD biomarkers and outcome was gathered over the last 35 years and has been published in multiple systematic reviews and meta-analysis (D'Aiuto, Orlandi, & Gunsolley, 2013; Janket, Baird, Chuang, & Jones, 2003; Khader, Albashaireh, & Alomari, 2004; Scannapieco, Bush, & Paju, 2003). Most of the interventional trials focused on surrogate markers of CVD and only a small amount on CVD outcomes. Endothelial dysfunction, as one of the most noticed surrogate markers can predict adverse CVD outcomes(Schächinger, Britten, & Zeiher, 2000). Positive effects on the endothelial function, assessed via changes in the flow-mediated dilation in the brachial artery following

periodontal treatment could be observed in an interventional study with 30 periodontitis patients and 31 periodontal healthy controls (Seinost et al., 2005). The significant improvement of flow-mediated dilation (p=0.003) was accompanied by a significant decrease in CRP concentrations (p=0.026). Interestingly shortly after this study another interventional trial was published with 120 patients with periodontitis (Tonetti et al., 2007). 61 underwent intensive periodontal treatment and flow-mediated dilation significantly improved on the long – term, after 6 months. The authors showed that intensive periodontal treatment resulted in an acute, short – term systemic inflammation (high CRP) and endothelial dysfunction.

To assess the risk for CVD mortality or morbidity randomized clinical trials (RCT) with periodontal therapy to prevent cardiovascular events are needed. A multicenter feasibility study (Beck, James D. et al., 2008) could not show any differences in CVD events rate between the periodontal treatment group and the control group. Since there are no RCT's concerning this scientific topic, no conclusion can be done to date.

Treatment duration in our study groups PT1 and PT2 took maximum nine days and were completed in maximum four sessions. Similar to the role model/feasibility study (Couper et al., 2008) the treatment was supposed to be finished within two months of randomization. One of the biggest differences of our study to the PAVE study were the dental inclusion criteria, which were stricter in our study, i.e. twelve vs. six natural teeth (including third molars), > 5mm PD vs ≥ 4mm PD. This design may reflect more a potential periodontal inflammation and may have more supposed influence on a systemic way. No emphasis was laid on the smoking status of the patients for stratification of our study subjects, which is a minor disadvantage since it is well known, that smoking impairs treatment outcome. One other disadvantage in our study is the patient's uncertainty and/or concomitant general health status and/or their daily medication intake. A lot of patients had a variety of concomitant medical problems like type 2 diabetes or heavy smoking. Most of the study participants attached no great importance to their health in general respective disease control and the urgency of dealing with problems or risk factors. The awareness that many of them can easily be solved or improved – like periodontal disease or smoking cassation – was often not given. Even if they fully understood the consequences and implications of their health status, a certain kind of resignation was noticeable. The PAVE and our study have a lack of consistency in supportive periodontal treatment in terms of regularity. Recruitment was, like in the PAVE study, the biggest challenge we had to face with. Patients with at least one remaining natural tooth were asked from the Division of Angiology to show up at the Department for Dental Medicine and Oral Health and since there was not always a patient transfer with an ambulance transport, possible excessive

persuasive efforts were often needed. But given these facts, that the patients had to switch locations between the study facilities quite much, the drop-out rates in all three groups are very low (= 5,55%).

Since the PAVE study intervention was conducted without the additional use of antiseptics like Chlorhexidine or adjuvant systemic antibiotics, comparability in terms of microbiological or inflammatory status are not reasonable. To detect smaller numbers of even non – viable periodontal pathogens, PCR techniques are a reliable and more precise than detection by culture technique (Boutaga, Van Winkelhoff, Vandenbroucke-Grauls, & Savelkoul, 2006). The used paper point sampling method seems to be suitable for microbiological diagnostics since sampling via curettes and transferring again by a paper point in a sterile container is more complex and brings no major advantages (Jervøe-Storm, AlAhdab, Koltzscher, Fimmers, & Jepsen, 2007). The number of studies who compare subgingival samples before and after treatment via PCR techniques are rare.
A research group in Leipzig, Germany conducted a comparison of real-time PCR and DNA-strip technology in microbiological evaluation of periodontitis treatment in 25 patients with chronic periodontitis defined by CAL ≥ 5mm at more than 30% of sites and an age of ≥ 35 years and a mean age, 50.6 years (Eick, Straube, Guentsch, Pfister, & Jentsch, 2011) with similar protocol like our study. In addition to our baseline, three - and twelve months subgingival plaque sampling, they added another time point at six months. A. actinomycetemcomitans did not differ significantly between the different sampling times but the other major pathogens Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola differed clearly between the time points. To compare these results logically only the PT2 group is admissible. From these 28 patients no significant difference in composition of subgingival periodontal pathogens could be shown at any time point ($p > 0.05$). Only in the PT1 group we could detect a significant decrease ($p < 0.05$) of periodontal pathogens Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Campylobacter rectus, Eubacterium nodatum and Capnocytophaga sp. These results are also consistent with a vitro study, where the combination of Amoxicillin and Metronidazole showed the strongest effect on numbers of putative pathogens (Belibasakis & Thurnheer, 2014). The combination shows also the most potential effect than any other antibiotic regimen targeting periodontal pathogens (Mombelli, 2012).

MMP-8 is discussed in the literature as a point-of-care biomarker in periodontitis and cardiovascular diseases (Sorsa et al., 2011) and can be detected in oral fluids like gingival crevicular fluid (GCF), peri-implant sulcular fluid (PISF), mouth – rinse and saliva as well

as in serum and plasma. Oral fluids can be collected non – invasively and are useful diagnostic fluids for oral related diseases. Since many inflammation markers are detectable in saliva, like IL-1β, IL-6, IL-1, IL-8, (TNF-α) and MMP-8 and -9, monitoring salivary biomarkers for oral and systemic diseases could become a more and more important complement to clinical findings (Rathnayake et al., 2013). Especially elevated MMP-8 concentrations and periodontitis have a significant association for both clinical (higher PD) and radiographic (RBL) parameters (Gursoy et al., 2013). Our findings of initially higher MMP-8 concentrations in GCF and decreasing levels with lesser inflammation in both therapy groups ($p < 0.05$) are consistent with the literature (Reinhardt et al., 2010). Given the fact that with no therapy in the CG the levels of MMP-8 increased, detection of MMP-8 in GCF as a biomarker for destruction of periodontal attachment and alveolar bone loss seems to be a feasible method. The CG and the PT2 group had almost the same levels 12 months after baseline examination (19.5 ng/ml and 21.6 ng/ml).
The PT1 group showed the most decrease of MMP-8 concentration and stayed after 12 months at the very low post treatment level (9.4 ng/ml).

In the beginning of the new millennium a number of publications from the group of the Interleukin Genetics, Inc., San Antonio, Texas, USA concerning the association of IL-1 gene variations with periodontitis was issued. The study (and commercial) group postulated, that the composite IL-1 genotype is significantly associated with the severity of adult periodontitis. Further they highlight IL-1 genotyping and smoking history as objective risk factors for periodontal disease (McDevitt et al., 2000). Since chronic inflammatory diseases have many risk factors in common, studies for investigating periodontal disease in patients with concomitant systemic diseases with different IL-1genotypes were evaluated. IL-1 genotypes and the association between periodontitis and CVD were also researched and hypothetical models were presented to explain the link of IL-1 genetic factors (Kornman et al., 1999). In a cohort of 100 diabetic patients 66% showed periodontal destruction, defined as two or more teeth with CAL ≥ 5 mm (Guzman, Karima, Wang, & Van Dyke, 2003). Genotyping of IL-1A (+4845), IL-1B (+3954), IL-1B (–511), and IL-1RN (+2018) polymorphisms was conducted via finger – stick blood samples. Despite the high prevalence of periodontitis, associations of genotype with clinical findings was limited since the prevalence of some IL-1 gene polymorphisms were low. Even though allele 1 at IL-1B (–511) and IL-1B (+3954) was overrepresented among diabetics with periodontal disease it reached no statistical significance. In 2005 a group of researchers at the University of Chile, Santiago, Chile performed a case – control study of 330 cases of periodontitis patients and 101 healthy control subjects (López, Jara, & Valenzuela, 2005).

Both groups were aged 20 to 48 years, which is a quite young cohort given the fact, that they were categorized having initial, moderate, or severs periodontitis according to the number of sites with CAL ≥3 mm. Like in our study IL-1A-889 and IL-1B+3954 evaluation via PCR amplifications were performed. Individuals carrying the positive genotype have significantly greater risk for developing periodontitis. Recently a study group from the Sri Ramachandra University, Chennai, India recruited 200 gingival healthy and 200 periodontitis patients to determine the association between single nucleotide polymorphisms in in IL-1B (−511, +3954), IL-1A (−889, +4845), and the variable number of tandem repeats (VNTRs) polymorphism in the IL-1RN gene (Lavu et al., 2015). They could show that a higher proportion of the variant alleles were observed in the chronic periodontitis group for all the SNPs examined ($p<0.023$). Since there are differences of the frequency of IL-1 gene – polymorphisms due to ethnicity, comparison with studies from different continents or regions is questionable. Subjects of Chinese heritage have been found to have 2.3% of positive IL-1 genotype (Armitage et al., 2000) whereas up to 14% of an African – American control population was tested positive for IL-1 genotype compared to only 8% in localized form of aggressive periodontitis (Walker et al., 2000).

In our study population no findings in terms of higher percentage of IL-1 positive genotype can be shown. Limitations are certainly the small number of patients and the fact that all of our patients had moderate to severe forms of periodontitis and concomitant PAD. No healthy controls were compared. The high prevalence of cigarette smoking possibly concealed the IL-1 genotyping risk factor in our cohort.

7. CONCLUSION

Periodontitis is one of the most prevalent inflammatory diseases worldwide. Since it has been named also a “silent disease”, the attention to the prevalence and the impact on the oral health itself was not sufficient. In the past 2 decades a number of studies laid emphasis on intensive research in the growing discipline of periodontal medicine.

This study aimed to investigate two different questions. How high is the prevalence of periodontitis in a cohort with PAD, and what are the impacts of periodontal therapy on this specific population with concomitant systemic disease? Although we knew from epidemiological studies that periodontitis is frequent in patients with systemic diseases, the high prevalence of periodontitis cases besides the fact that most of the screening patients were excluded due to low number of teeth (<12) were both astonishing and alarming. The widespread abuse and associated massive burden of cigarette smoking in this cohort is also a very worrying aspect and big effort should be done to reach smokers on a rational and empathic level.

Like in the feasibility study (PAVE), patient recruitment was one of the biggest challenges throughout the study period, since the appointments had to be kept very strict, especially in the treatment groups. Coordination of the different examinations like internal medicine, dental, radiology, etc. was only possible because of large input from all participants; investigators and patients.

The impact of the periodontal treatment on clinical parameters (PISA), microbiological (via PCR, only in PT1) and on a biomarker level (MMP-8) could be shown in the treatment groups. Since the systemic antibiotics in PT1 and the adjunctive antiseptic therapy in both groups was administered at home, no verification on compliance could be done. It has been shown that periodontal treatment in patients with PAD is safe and that the use of antibiotics to reduce the inflammatory burden was superior than no adjuvant systemic antibiotic treatment.

Whether patients with PAD have a benefit on a vascular level remains an open question.

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