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ASSESSMENT OF THE ASSOCIATION BETWEEN CEREBRAL SMALL VESSEL DISEASE, PERIODONTAL DISEASE AND CARDIOVASCULAR RISK FACTORS

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ASSESSMENT OF THE ASSOCIATION BETWEEN CEREBRAL SMALL VESSEL DISEASE, PERIODONTAL DISEASE AND CARDIOVASCULAR RISK FACTORS

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Assessment of the association between cerebral small vessel disease, periodontal disease and cardiovascular risk factors

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RESUMO

Introdução: A doença periodontal e a doença cardiovascular são entidades prevalentes, que frequentemente coexistem, e com uma via pró-inflamatória comum. A doença dos pequenos vasos cerebrais refere-se a um conjunto de processos patológicos, crónicos e progressivos, que afetam as arteríolas, capilares e pequenas veias responsáveis pela vascularização da substância branca e estruturas profundas da substância cinzenta. As evidências mais recentes parecem sugerir que as infeções orais crónicas, como a periodontite, contribuem para a progressão da doença dos pequenos vasos cerebrais. Neste estudo, avaliamos a relação entre a doença periodontal, as hiperintensidades da substância branca cerebral e os marcadores de risco cardiovascular pró-inflamatórios raramente estudados neste contexto.

Material e Métodos: A amostra consistiu em 43 doentes, com idades compreendidas entre os 38 e os 82 anos, sem antecedentes cardiovasculares, hipertensos, 42% do género feminino e 50% diabéticos. Foi estudada a associação entre a média da profundidade de sondagem (MPS), média da perda de inserção (MPI), hemorragia pós-sondagem (HPS) e área total de superfície periodontal inflamada (ATSPI) com os marcadores pró-inflamatórios de risco cardiovascular (razão neutrófilo-linfócito (RNL), pressão arterial ambulatória 24h, risco cardiovascular global, ingestão diária de sal e velocidade de onda de pulso (VOP)) e com as hiperintensidades da substância branca (HSB).

Resultados: HPS, MPS e MPI correlacionaram-se significativamente com a ingestão elevada de sal, estimativa de risco cardiovascular global, pressão arterial sistólica noturna (nPAS) e ATSPI. Além disso, foi encontrada uma correlação positiva entre HPS, RNL e VOP. A análise de regressão multivariada mostrou uma relação independente entre HPS e nPAS, ingestão diária de sal e RNL. A análise dos dados também revelou uma correlação inversa não significativa entre MPS, HPS, ATSPI e HSB. Foi encontrada uma correlação positiva não significativa entre MPS e HSB.

Conclusões: No nosso estudo, e com a nossa amostra, HPS e ATSPI associaram-se a um marcador de inflamação circulatório (RNL). MPS e MPI, mas particularmente HPS, também se relacionaram com o risco cardiovascular global e com marcadores pró-inflamatórios tais como a hipertensão e o consumo de sal. Menores valores de MPS, HPS e ATSPI correspondem a um maior número de HSB. Em sentido inverso, a maiores MPI estão associadas um maior número de lesões de substância branca.

Palavras-Chave: Doença Periodontal, Inflamação, Sal, Hipertensão, Risco Cardiovascular, Velocidade Onda Pulso, Doença Pequenos Vasos Cerebrais, Substância Branca

ABSTRACT

Introduction: Periodontal disease and cardiovascular disease are prevalent entities that often coexist, with a common pro-inflammatory pathway. Cerebral small vessel disease is a chronic progressive disorder of arterioles, capillaries and small veins supplying the brain white matter and deep structures of gray matter. Latest evidence seems to suggest that chronic oral infections such as periodontitis contribute to cerebral small vessel disease progression. In this study we evaluated the relationship between periodontal disease, brain white matter hyperintensities and pro-inflammatory cardiovascular risk markers rarely studied in this context.

Materials and Methods: 43 patients, aged between 38-82 without previous cardiovascular events, hypertensive, 42% female and 50% diabetic were evaluated. An association between mean probing depth (MPD), mean attachment level (MAL), bleeding on probing (BOP), and total periodontal inflamed surface area (TPISA) with cardiovascular disease factors and inflammatory promoters (neutrophil-to-lymphocyte ratio (NLR), 24h ambulatory blood pressure, global cardiovascular risk, daily salt intake, pulse wave velocity (PWV)) and white matter hyperintensities (WMH) was studied.

Results: BOP, MPD and MAL correlated significantly with high salt intake, global cardiovascular risk estimation, nighttime systolic blood pressure (nSBP) and TPISA. Also, a positive correlation between BOP, NLR and PWV was found. Multivariate regression analysis showed an independent relationship between BOP and nSBP, salt intake and NLR. Data analysis also revealed an inverse non-significant correlation between MPD, BOP, TPISA and WMH. A positive non-significant correlation was found between MAL and WMH.

Conclusions: In our study and within our sample BOP and TPISA were associated with a circulatory inflammation marker (NLR). MPD and MAL but particularly BOP

were also related to global CV risk and pro-inflammatory factors, such as hypertension and salt intake. Lower values of MPD, BOP and TPISA were associated with higher WMH. Contrarily MAL was positively associated with WMH.

Keywords: Periodontal Disease, Inflammation, Salt Intake, Hypertension, Cardiovascular Risk, Pulse Wave Velocity, Cerebral Small Vessel Disease, White Matter

“There are no shortcuts to any place worth going.”

Beverly Stills

À minha mulher
pelo compromisso
pela dedicação
pela vida.

Aos meus dois filhos
pelo que me ensinam
todos os dias.

Aos meus Pais
pelo exemplo
pelos valores.

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LIST OF ABBREVIATIONS

AAP	American Academy of Periodontology
ABP	Ambulatory Blood Pressure
ASCVD	Atherosclerotic Cardiovascular Disease
BMI	Body Mass Index
BOP	Bleeding on Probing
BP	Blood Pressure
CAA	Cerebral Amyloid Angiopathy
CAL	Clinical Attachment Loss
CEJ	Cemento-Enamel Junction
CSF	Cerebrospinal Fluid
CSVD	Cerebral Small Vessel Disease
CV	Cardiovascular
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
FPG	Fasting Plasma Glucose
HbA1C	Glycated Hemoglobin
HDL	High-Density Lipoprotein
JE	Junctional Epithelium
LDL	Low-Density Lipoprotein
LDL-c	Low-Density Lipoprotein-Cholesterol
MAL	Mean Attachment Level
MPD	Mean Probing Depth
MRI	Magnetic Resonance Image
NDR	Night-to-Day Ratio
NLR	Neutrophil-Lymphocyte Ratio
OBP	Office Blood Pressure
PD	Pocking Depth
PESA	Periodontal Epithelial Surface Area
PG	Porphyromonas Gingivalis
PISA	Periodontal Inflamed Surface Area
PWV	Pulse Wave Velocity

SBP	Systolic Blood Pressure
SCORE	Systematic Coronary Risk Evaluation
TPISA	Total Periodontal Inflamed Surface Area
WBC	White Blood Cells
WHO	World Health Organization
WMH	White Matter Hyperintensities

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1. INTRODUCTION

1. INTRODUCTION

Gingival and periodontal diseases are not recent discoveries. Paleopathological studies have indicated that diseases of the gums have afflicted humans since the beginnings of history while dental caries, for example, arose with the development of the civilizations, related to dietary habits. One of the earliest cases of periodontitis known was observed in Egyptian mummies, about 4000 years ago[1]. The Greek physician Hippocrates born in 460 B.C. in the island of Kos, considered the father of modern medicine, gave a fundamental contribution to medicine through the collection of manuscripts, known as the Hippocratic Collection which are the oldest written sources of western medicine that cover all aspects of medicine at that time[2]. Hippocrates discussed the etiology and pathogenesis of different forms of periodontal disease, including the situation when the gums were bleeding or rotten[3]. However, the first directed therapy to periodontitis is attributed to Pierre Fauchard while providing the first discussion of periodontal pathology where he recommended scaling of the teeth with special instruments to remove calculus and also mouthwashes, dentifrices and the splinting of loose teeth[4]. In what concerns to periodontal diseases classification, an English physiologist and surgeon with scientific interests named John Hunter, in 1802 proposed a classification that identified inflammatory processes in the gingiva as important factors in the destruction of the alveolar bone[5].

In the 19th century, dental treatments where in its vast majority limited to dental extractions and periodontal disease was considered incurable. In an article published in 1882 by Riggs the author considered that calculus deposits and other foreign bodies that made the tooth surface rough were the cause of the diseases of the gingiva and the bone, so “alveolar pyorrhea” could be cured by removal of these accretions. Riggs seems to have been the first practitioner to limit his practice to the treatment of periodontal disease being an important contributor to the development of clinical periodontics[6].

Emerging evidence suggests that periodontal disease may be associated with various

systemic conditions as described in recent meta-analyses of observational studies, such as increased risk of chronic kidney disease[7], rheumatoid arthritis[8] and liver cirrhosis[9], in individuals with periodontal disease, especially those with severe periodontitis.

Cardiovascular diseases have been the leading cause of death in recent years having already overcome infectious diseases. Also, the prevalence of CV disease in young people has increased drastically, being expected that its prevalence will continue to grow as average life expectancy is also increasing. According to the World Health Organization, chronic noncommunicable diseases, such as CV diseases, cancer, chronic respiratory diseases and diabetes, constitute about 70% of worldwide causes of death[10].

Periodontal and cardio-cerebrovascular disease are both important and high prevalent health conditions. The development of several systemic diseases such as endocarditis posterior to dental procedures, have been related to poor oral health. Therefore, periodontal disease has emerged as a potential important risk factor for cerebrovascular disease and the possible relation between periodontitis and the higher risk of cerebrovascular events, such as strokes, reinforce the importance of oral health in general health.

1.1. Periodontal disease

Periodontitis is a multifactorial chronic inflammatory disease, characterized by the inflammation of the teeth supportive tissues (periodontal ligament, cementum and alveolar bone). If untreated, can cause irreversible damage of those structures with consequent tooth loss. It is a high prevalence disease (45-50%) and its most severe form affects 10-15% of the adult population[11]. Gingivitis is the mildest form of periodontal disease, and it is characterized by the inflammation of the soft tissue most commonly due to the accumulation of bacteria, without apical migration of the junctional epithelium. It is a reversible, nondestructive disease that does not involve loss of periodontal tissues and is identified clinically as inflammation of the gingiva

causing one or more soft tissue changes including redness, edema, increase in thickness, ulceration, or bleeding[12]. Gingivitis is commonly painless, rarely leads to spontaneous bleeding, and is often characterized by subtle clinical changes, resulting in most patients being unaware of the disease or unable to recognize it[13].

Gingivitis can also be classified according to the periodontium condition:

- Gingivitis on an intact periodontium.
- Gingivitis on a reduced periodontium in a non-periodontitis patient. (e.g., recession, crown lengthening)
- Gingival inflammation on a reduced periodontium in a successfully treated periodontitis patient.

Accordingly to the Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions[14], there are, generally, two categories of gingival disease:

Dental Plaque Biofilm-Induced Gingivitis

This category of gingivitis is caused by the interaction between the bacteria present in the dental plaque biofilm and the immune/inflammatory response causing an inflammatory lesion which does not affect the periodontal attachment. It is reversible through the reduction of the dental plaque biofilm both at gingival margin and apically, as it does not extend beyond mucogingival junction. On the other hand, the amount of plaque accumulation necessary to cause disease, its severity and progression rate, varies between individuals because of the presence of local/predisposing risk factors, and systemic/modifying risk factors.

Local/Predisposing Risk Factors

These factors are those that facilitate plaque accumulation at a specific site through preventing its removal during common hygiene practices, and/or creating a biological niche that facilitates plaque accumulation[15]. These include:

a) Dental plaque biofilm retention factors – Tooth and root anatomy, restorative and endodontic considerations, and other tooth-related factors[16] can influence the severity of plaque induced gingivitis because they facilitate plaque accumulation at and apical to the gingival margin, enabling biofilm adherence and maturation thereby increasing the difficulty of mechanical plaque removal.

b) Oral dryness – Oral dryness is a clinical condition often associated with symptoms of xerostomia due to hyposalivation[17]. The lack of salivary flow, availability, or changes in quality of saliva lead to reduced cleansing of tooth surfaces and, consequently, difficulty in dental plaque biofilm removal and enhanced gingival inflammation. It may also cause progressive dental caries, taste disorders, halitosis, and inflammation of the oral mucosa, tongue and gingiva[15].

Systemic Risk Factors (modifying factors)

These risk factors include the characteristics present in an individual, which negatively influence the immune-inflammatory response to a given dental plaque biofilm burden, causing exaggerated or "hyper" inflammation.

a) Smoking – Causes profound effects on the gingival tissues being one of the major behavioral risk factors for periodontitis[18]. Indirect smokers absorb the smoke through the pulmonary alveolar epithelium, entering the systemic circulation. On the other hand, direct exposure of inhaled cigarette smoke to periodontal tissues causes vasoconstriction of the periodontal microvasculature and gingival fibrosis, which can mask clinical signs of gingivitis, such as bleeding on probing[15,19,20].

b) Metabolic factors – High levels of glucose (hyperglycemia associated or not with diabetes mellitus) induce mitochondrial stress which activate proinflammatory cascades leading to proinflammatory events [21].

c) Nutritional factors – Severe Vitamin C deficiency is a nutritional deficit that has well-documented effects on the periodontium and usually has characteristics that are similar to plaque-induced gingivitis[15]. It results in compromised antioxidant micronutrient defenses to oxidative stress having a negative impact on collagen synthesis, which causes loss of tonus in capillary blood vessel walls and consequent propensity to enhanced gingival bleeding[22].

d) Pharmacological agents – It is known that several medications affect the gingival tissues[23]. These include oral contraceptives in high dosage, antiepileptic drugs such as phenytoin and sodium valproate, certain calcium channel–blocking drugs and immunoregulating drugs like cyclosporine[24].

e) Elevations in sex steroid hormones – It has been demonstrated that steroid hormones (androgens, estrogens and progestins) can affect the periodontal tissues[25]. Thus, puberty, pregnancy and oral contraceptives intake may increase the gingival inflammatory response even in the absence of excessive plaque accumulation. Nowadays, oral contraceptive steroid concentrations are much lower and it is known that current formulations of oral contraceptive do not induce the clinical changes in gingiva that were reported with high dose contraceptives[26].

f) Hematological conditions – excessive inflammation, and other oral manifestations is found in some blood malignancies such as leukemia even in the presence of relatively low levels of plaque. 17,7% of the patients with acute leukemia present gingival bleeding as the initial oral sign and/or symptom whereas it occurs in 4.4% of the patients with chronic leukemia[27]. Gingival enlargement has also been reported, as a result of the infiltration of leukemic cells, initially at the interdental papilla and then both at the marginal and attached gingiva[28].

Non-Dental Plaque-Induced Gingivitis

Inflammatory gingival disease can also be caused by systemic disorders, with no direct relation with the presence of dental plaque biofilm, although they can be aggravated by plaque accumulation. A classification based on the etiology of the lesions was proposed[29], considering the following conditions:

- Genetic/Developmental disorders
- Specific infections
- Inflammatory and immune conditions and lesions
- Reactive processes
- Neoplasms
- Endocrine, nutritional and metabolic diseases
- Traumatic lesions
- Gingival pigmentation

Gingivitis has unclear significance as a predictor of future periodontal tissue loss[30], however is a major risk factor and a necessary pre-requisite for periodontitis. Therefore, management of gingivitis is a primary preventive key strategy for periodontal disease.

Periodontitis refers to the inflammation of the periodontium that is accompanied by apical migration of the junctional epithelium, allowing the migration of bacteria along the root surface leading to destruction of the connective tissue attachment and alveolar bone loss[18,19]. Although gingival inflammation arises from the presence of bacterial biofilm, the initiation and progression of periodontitis depends on a modification of oral microbiome which is, on its turn, influenced by certain systemic factors such as smoking and systemic diseases. This makes some individuals more prone to develop this condition, also influencing the severity of the disease[31].

Classification of periodontitis has represented a challenge since the dissimilar clinical presentations could represent distinctive diseases or just variations of the same disease with different phenotypes[32].

The American Academy of Periodontology (AAP) has adopted several classification systems from the year 1977 to 1989[33], which are summarized in the table 1.

1977	1986	**1989**
I. Juvenile Periodontitis	**I.** Juvenile Periodontitis	**I.** Early-Onset Periodontitis
II. Chronic Marginal Periodontitis	a. Prepuberal	a. Prepuberal Periodontitis
	b. Localized juvenile periodontitis	1. Localized
	c. Generalized juvenile periodontitis	2. Generalized
	II. Adult Periodontitis	b. Juvenile Periodontitis
	III. Necrotizing Ulcerative Gingivo-Periodontitis	1. Localized
	IV. Refratory Periodontitis	2. Generalized
		c. Rapidly progressive
		II. Adult Periodontitis
		III. Necrotizing Ulcerative Periodontitis
		IV. Refractory Periodontitis
		V. Periodontitis Associated with Systemic Disease

Table 1. Evolution of the AAP periodontal disease classification system (AAP 1989).

Although the 1989 classification represented an improvement over the previous classifications, mainly because it considered the implication of systemic diseases on periodontitis, it still had several limitations – indefinite boundaries between categories which led to overlapping, a gingival disease category was not included, inaccurate classification criteria and also dubious information regarding rate of progression and onset of the disease[33].

Therefore, in 1999 a new classification system was proposed[34] and accepted by the AAP (Table 2-5). One of the most significant changes included the addition of a detailed section on gingival diseases (dental plaque-induced/not associated with dental plaque). Another important change was the discontinuation of terms related to age (chronic periodontitis instead of adult periodontitis) and related to onset and rate

of progression (for example early onset periodontitis was replaced by aggressive periodontitis).

The category of refractory periodontitis was eliminated, because of the great variety of situations that can lead to a non-responsive therapy. So, it was considered that all forms could be classified as refractory if they don´t respond to the treatment.

I. GINGIVAL DISEASES
A. Dental Plaque-Induced Gingival Diseases
1.Gingivitis associated with dental plaque only
a. without other local contributing factors
b. with local contributing factors
2. Gingival diseases modified by systemic factors
a. associated with the endocrine system
1) puberty associated gingivitis
2) menstrual cycle-associated gingivitis
3) pregnancy-associated
a) gingivits
b) pyogenic granuloma
4) diabetes mellitus-associated gingivits
b. associated with blood dyscrasias
1) leukemia-associated gingivitis
2) other
3. Gingival diseases modified by medications
a. drug-influenced gingival diseases
1) drug-influenced gingival enlargements
2) drug influenced gingivitis
a) oral contraceptive-associated gingivitis
b) other
4. Gingival diseases modified by malnutrition
a. ascorbic acid-deficiency gingivitis
b. other

Table 2. Classification of periodontal diseases and conditions (Armitage 1999).

I. GINGIVAL DISEASES
B. Non-Plaque-Induced Gingival Lesions
1. Gingival diseases of specific bacterial origin
a. *Neisseria gonorrhea*-associated lesions
b. *Treponema pallidum*-associated lesions
c. Streptococcal species-associated lesions
d. other
2. Gingival diseases of viral origin
a. herpesvirus infections
1) primary herpetic gingivostomatitis
2) recurrent oral herpes
3) varicella-zoster infections
b. Other
3. Gingival diseases of fungal origin
a. Candida-species infections
1) generalized gingival candidiasis
b. linear gingival erythema
c. histoplasmosis
d. other
4. Gingival diseases modified by malnutrition
a. ascorbic acid-deficiency gingivitis
b. other
5. Gingival manifestations of systemic conditions
a. mucocutaneous disorders
1) lichen planus
2) pemphigoid
3) pemphigus vulgaris
4) erythema multiforme
5) lupus erythematosus
6) drug-induced
7) other
b. allergic reactions
1) dental restorative materials
a) mercury
b) nickel
c) acrylic
d) other
2) reactions attributable to
a) toothpastes/dentifrices
b) mouthrinses/mouthwashes
c) chewing gum additives
d) foods and additives
3) other

Table 3. Classification of periodontal diseases and conditions (Armitage 1999) (continuation).

I. GINGIVAL DISEASES
B. Non-Plaque-Induced Gingival Lesions
6. Traumatic lesions (factitious, iatrogenic, accidental)
a. Chemical injury
b. physical injury
c. thermal injury
7. Foreign body reactions
8. Not otherwise specified (NOS)
II. CHRONIC PERIODONTITIS
A. Localized
b. Generalized
III. AGGRESSIVE PERIODONTITIS
A. Localized
B. Generalized
IV. PERIODONTITIS AS A MANIFESTATION OF SYSTEMIC DISEASES
A. Associated with hematological disorders
1. Acquired neutropenia
2. Leukemias
3. Other
B. Associated with genetic disorders
1. Familial and cyclic neutropenia
2. Down syndrome
3. Leukocyte adhesion deficiency syndromes
4. Papillon-Lefevre syndrome
5. Chédiak-Higashi syndrome
6. Histiocytosis syndromes
7. Glycogen storage disease
8. Infantile genetic agranulocytosis
9. Cohen syndrome
10. Ehlers-Danlos Syndrome (Types IV and VIII)
11. Hypophosphatasia
12. Other
C. Not otherwise specified (NOS)

Table 4. Classification of periodontal diseases and conditions (Armitage 1999) (continuation).

V. NECROTIZING PERIODONTAL DISEASES
A. Necrotizing ulcerative gingivitis B. Necrotizing ulcerative periodontitis
VI. ABSCESSES OF THE PERIODONTIUM
A. Gengival abscess B. Periodontal abscess C. Pericoronal abscess
VII. PERIODONTITIS ASSOCIATED WITH ENDODONTIC LESIONS
A. Combined periodontic-endodontic lesions
VIII. PERIODONTITIS AS A MANIFESTATION OF SYSTEMIC DISEASES
A. Localized tooth-related factors that modify or predispose to plaque-induced gingival diseases/periodontitis 1. Tooth anatomic factors 2. Dental restoration/appliances 3. Root fractures 4. Cervical root resorption and cemental tears B. Mucogingival deformities and conditions around teeth 1. Gengival/soft tissue recession a. facial or lingual surfaces b. interproximal (papillary) 2. Lack of keratinized gingiva 3. Decreased vestibular depth 4. Aberrant frenum/muscle position 5. Gingival excess a. pseudopocket b. inconsistent gingival margin c. excessive gingival display d. gingival enlargement 6. Abnormal color C. Mucogingival deformities and conditions on edentulous ridges 1. Vertical and/or horizontal ridge deficiency 2. Lack of gingival/keratinized tissue 3. Gingival/soft tissue enlargement 4. Aberrant frenum/muscle position 5. Decreased vestibular depth 6. Abnormal color D. Occlusal trauma 1. Primary occlusal trauma 2. Secondary occlusal trauma

Table 5. Classification of periodontal diseases and conditions (Armitage 1999) (continuation).

Periodontitis as a manifestation of systemic diseases was maintained although some clinical conditions such as diabetes are no longer considered in this category. Necrotizing ulcerative periodontitis was replaced by necrotizing ulcerative diseases and includes necrotizing ulcerative gingivitis and necrotizing ulcerative periodontitis, since investigators were unsure if these are distinct clinical entities and because both can be manifestations of the same systemic condition (e.g., HIV).

Periodontal abscess was added as a new category, although they can be found in many forms of periodontitis, as these clinical conditions are often very challenging in terms of diagnosis and treatment.

A category on Periodontic-Endodontic lesions was included and a category on Developmental or acquired deformities and conditions that doesn´t refer to separate diseases but to several factors that can significantly influence the susceptibility to periodontal disease or the response to treatment.

A clear definition of periodontal health like in any other condition is crucial, in first place, to adequately diagnose the disease and establish therapeutic goals.

It is also important for the investigators to study the prevalence in the population and the individual risk assessment.

A new classification system was proposed during the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions in order to overcome some limitations of the previous classification[34]. It was accepted that the main parameter for a gingivitis diagnosis would be bleeding on probing and that a gingivitis condition could revert to a healthy status while a periodontitis patient would keep the condition for the rest of his life[35].

Considering the current knowledge on periodontitis pathophysiology, a new classification arose, and three different forms were identified[36] :

1) Necrotizing periodontitis
2) Periodontitis as a manifestation of systemic disease
3) Periodontitis

This new classification[31] uses a **staging** and **grading** approach:

Staging has been used for many years in oncology and was recently discussed relatively to periodontal disease. It is categorized from stage I to IV and refers to the severity of the disease and complexity of the treatment, considering several factors such as clinical attachment loss, type and extent of bone loss, probing depth, presence and extent of angular bony defects and furcation involvement, tooth mobility and tooth loss due to periodontitis (Table 6). Therefore, the main goals of staging are to classify the severity and the extent of the disease (according to the tissue damage) and determine the factors that may complicate the management of the disease[31,36] .

Stage I - Refers to early stages of the disease that has progressed from a gingivitis condition.

Stage II - In this stage, periodontal disease has been established but is usually controlled by the standard therapeutic measures.

Stage III - In this stage, patients present deep periodontal lesions extending to the middle portion of the root. These lesions can be aggravated by intrabony defects, furcation involvement and previous history of tooth loss and, if not adequately treated, can cause tooth loss.

Stage IV - Refers to the more advanced stage of the disease, with deep periodontal lesions extending to the apical portion of the root that can lead to loss of the dentition.

Periodontitis Stage		Stage I	Stage I	Stage III	Stage IV
Severity	**Interdental CAL at site of greatest loss**	1 to 2mm	3 to 4mm	≥5mm	≥5mm
	Radiographic bone loss	Coronal third (<15%)	Coronal third (<15%)	Extending to mid-third of root and beyond	Extending to mid-third of root and beyond
	Tooth loss	No tooth loss due to periodontitis		Tooth loss due to periodontitis of ≤4 teeth	Tooth loss due to periodontitis of ≥5 teeth
Complexity	**Local**	Maximum probing depth ≤4 mm Mostly horizontal bone loss	Maximum probing depth ≤5 mm Mostly horizontal bone loss	In addition to stage II complexity: Probing depth ≥6 mm Vertical bone loss ≥3 mm Furcation involvement Class II or III Moderate ridge defect	In addition to stage III complexity: Need for complex rehabilitation due to: Masticatory dysfunction Secondary occlusal trauma (tooth mobility degree ≥2) Severe ridge defect Bite collapse, drifting, flaring Less than 20 remaining teeth (10 opposing pairs)
Extent and distribution	**Add to stage as descriptor**	For each stage, describe extent as localized (<30% of teeth involved), generalized, or molar/incisor pattern			

Table 6. Periodontitis **stage** (Tonneti et al 2018).

Grading provides information about the risk and rate of progression (considering previous disease history) and estimates the impact on the general condition of the patient, who is classified into grade A, B and C. It also considers individual risk factors such as smoking, diabetes or oral hygiene that can influence the outcome of the disease/treatment (Table 7 e 8). The objectives of grading a periodontal patient are to evaluate the risk of the progression of the disease and, consequently, adequate therapeutic measures and predicting the possible impact on systemic diseases (and vice-versa) [31,36].

Periodontitis Grade			**Grade A: Slow rate of progression**	**Grade B: Moderate rate of progression**	**Grade C: Rapid rate of progression**
Primary criteria	Direct evidence of progression	Longitudinal data (radiographic bone loss or CAL)	Evidence of no loss over 5 years	<2mm over 5 years	≥2mm over 5 years
	Indirect evidence of progression	% bone loss/age	<0.25	0.25 to 1.0	>1.0
		Case phenotype	Heavy biofilm deposits with low levels of destruction	Destruction commensurate with biofilm deposits	Destruction exceeds expectation given 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	Risk factors	Smoking	Non-smoker	Smoker <10 cigarettes/day	Smoker ≥10 cigarettes/day
		Diabetes	Normoglycemic/no diagnosis of diabetes	HbA1c < 7.0% in patients with diabetes	HbA1c ≥ 7.0% in patients with diabetes

Table 7. Periodontitis **grade** (Tonneti et al 2018).

Periodontitis Grade			Grade A: Slow rate of progression	Grade B: Moderate rate of progression	Grade C: Rapid rate of progression
Risk of systemic impact of periodontitis	Inflammatory burden	High sensivity CRP (hs CRP)	< 1mg/L	1 to 3 mg/L	> 3 mg/L
Biomarkers	Indicators of CAL/bone loss	Saliva, gingival crevicular fluid, serum	?	?	?

Table 8. Periodontitis **grade** (Tonneti et al 2018) (Continuation).

In general, **Grade A** refers to a slow rate of progression with no evidence of bone/clinical attachment loss (CAL); In **Grade B** patients, the rate of progression of the disease is moderate with less than 2 mm of bone/CAL loss over 5 years; In **Grade C** there is a rapid rate of progression with more than 2 mm of bone/CAL loss over 5 years.

Differential diagnosis of the three forms of the disease, is made by clinical history and signs and symptoms that can be identified by the clinician:

1) **Necrotizing periodontitis** has specific signs and symptoms - pain, fetid oral odor, rapid onset of the disease, ulceration of the gingival margin and loss of attachment[37,38]. Although uncommon, the disease can affect alveolar bone with osteonecrosis[39].

2) **Periodontitis as a direct manifestation of systemic diseases** is diagnosed by the presence of a rare systemic disease usually with an immunity component[34].

3) **Periodontitis** refers to the majority of the clinical situations that do not include the characteristics of necrotizing periodontitis neither are associated with a systemic disease.

Therefore, an accurate periodontitis diagnosis is not static and has to consider three different aspects[31]: In first place, the confirmation of tissue damage at two non-adjacent teeth - CAL/bone loss. Then, the form of the disease must be categorized - necrotizing periodontitis / periodontitis as a direct manifestation of systemic diseases / periodontitis and finally, staging and grading of the disease in order to classify the severity and the extent of periodontal damage and establish the rate of progression of the disease.

1.2. Cardiovascular Disease

Cardiovascular disease (CVD) affects the heart and the blood vessels and includes ischemic heart disease, cerebrovascular diseases, peripheral vascular disease and atherosclerotic vascular diseases which are the number one cause of death globally, accounting for approximately 30% of all deaths worldwide[40]. The increase in cardiovascular diseases is related to the developmental process as non-healthy lifestyles (e.g. obesity, smoking, physical inactivity) are associated with industrialized populations[41,42,43,44].

The Framingham Heart Study[35] revealed that 49% of men and 32% of women over 40 years old presented with clinical manifestations of coronary disease throughout life. Atherosclerotic vascular disease begins with the deposition of lipoproteins (e.g., LDL-c) in the inner lining of an artery, followed by an inflammatory process with accumulation of monocytes and lymphocytes[36]. Then, circulating monocytes attach to vascular endothelium. The activation of macrophage monocytes in blood vessels leads to the release of hydrolytic enzymes, cytokines, chemokines and growth factors that induce tissue damage, resulting in focal necrosis.

The circulating monocytes that reach the vascular wall through the endothelium, differentiate into macrophages which are transformed in foam cells filled with lipids. These cells are part of atheroma plaques who are responsible for the narrowing of vascular lumen. This narrowing combined with the atherosclerotic plaque rupture activate the platelet aggregation cascade and clotting, resulting in an occlusive or embolic thrombotic process typical of cardio-cerebrovascular events[45,46,47] .

Regarding the cardiovascular (CV) risk factors, they can be classified as behavioral (smoking, excessive alcohol ingestion, sedentarism and poor nutrition) psychosocial (stress, anxiety and depression) and biomedical. On its turn, biomedical risk factors can be subdivided in modifiable, such as obesity, dyslipidemia, diabetes, hypertension and renal disease. Non-modifiable risk factors include age, gender and family background. The higher the number of CV risk factors, the higher the risk for CVD[48].

1.3. Cerebral Small Vessel Disease

Cerebral small vessel disease (CSVD) is a chronic, progressive disorder of arterioles, capillaries and small veins supplying the white matter and deep structures of gray matter. It is the most common pathological neurological process and the attributable cause of 25% of strokes and more than doubles the odds of recurrent stroke. Furthermore, it contributes to 45% of dementia cases and to global functional decline[49,50,51]. Abnormalities affecting the structure and function of small vessels of the brain characterize this condition, with numerous neuroimaging and neurological manifestations[52]. CSVD encloses different sporadic and inherited diseases, resulting from a mixture of genetic and vascular risk factors[53]. Most prevalent forms of CSVD can be subdivided in amyloidal and non-amyloidal subtypes regarding the underlying pathophysiology. The amyloidal form includes the chronic degenerative disease cerebral amyloid angiopathy (CAA). The non-amyloidal form refers to CSVD often related with common vascular risk factors such as diabetes mellitus, hypertension, ageing and others[54].

Amyloidal cerebral small vessel disease

CAA is a common small vessel disease of the brain, mostly in the elderly[55] and characterized by the progressive deposition of amyloid-β protein in the walls of cerebral cortex and leptomeningeal small arteries, arterioles and capillaries, leading to vessel dysfunction and consequent intra-cerebral spontaneous hemorrhage[56,57]. Important morphological changes in the vessels include loss of smooth muscle cells, wall thickening and loss of compliance leading to breakable, fragile vessels predisposing to microaneurysm formation and leakage[58,59,57].

Non-amyloidal cerebral small vessel disease

Non-amyloidal CSVD is less specific and usually refers to hypertensive CSVD although is often related with a variety of risk factors such as diabetes mellitus and hypertension[60]. Pathological vascular changes are mainly arteriolosclerosis and atherosclerosis, being the first the most common, age-related, small vessel alteration in elderly brains. Not only hypertension and diabetes mellitus are known to aggravate this condition[61,62] but other conventional risk factors such as smoking, obesity and dyslipidemia have been considered to promote arteriolosclerosis[63].

Loss of smooth muscle cells from the tunica media, degeneration of internal elastic lamina, proliferation of fibroblasts, deposits of fibro-hyaline material and collagens, thickening of the vessel wall, formation of microatheroma, and narrowing of the lumen are consequences of cerebral small vessel atherosclerosis[54], contributing to reduce cerebral blood flow and subsequent chronic cerebral hypoperfusion[64].

1.3.1. Neuroimaging in Cerebral Small Vessel Disease

Neuroimaging assumes a key role in the diagnosis and characterization of CSVD since cerebral lesions caused by small vessel changes have been validated as markers of CSVD[54].

Because the terms and definitions of these cerebral lesions have varied considerably between different studies[65,66], the proposed terminology for neuroimaging features regarding CSVD includes[67]:

Recent small subcortical infarct

Neuroimaging evidence of recent infarction in the territory of one perforating arteriole, with imaging features or clinical symptoms consistent with a lesion occurring in the previous few weeks.

Lacune of presumed vascular origin

A round or ovoid, subcortical, fluid-filled cavity (signal similar to CSF) from 3 mm to about 15 mm in diameter, consistent with a previous acute small subcortical infarct or hemorrhage in the territory of one perforating arteriole.

White matter hyperintensity of presumed vascular origin

Signal abnormality of variable size in the white matter that shows the following characteristics: hyperintensity on T2-weighted images such as fluid-attenuated inversion recovery, without cavitation (signal different from CSF). Lesions in the subcortical grey matter or brainstem are not included in this category unless explicitly stated. If deep grey matter and brainstem hyperintensities are also included, the collective term should be subcortical hyperintensities. (Fig. 1 and Fig. 2)

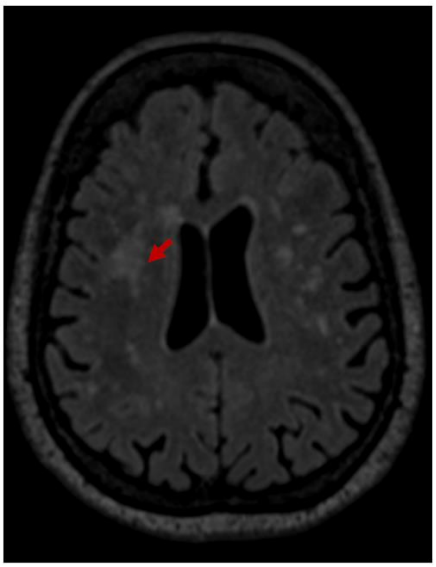

Figure 1. T2-weighted brain MRI from participating patient showing semiovale centrum confluent white matter hyperintensities (WMH).

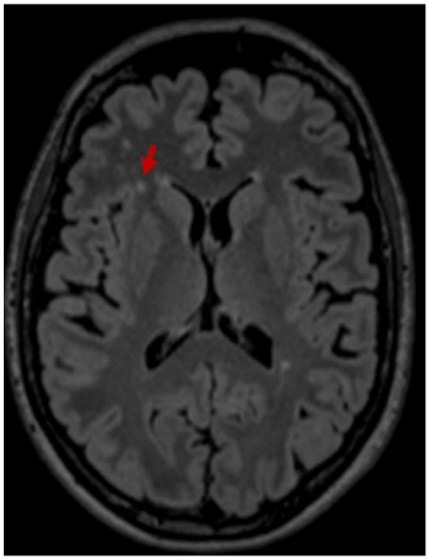

Figure 2. T2-weighted brain MRI from participating patient showing low white matter hyperintensities (WMH) load.

Perivascular space

Fluid-filled spaces that follow the typical course of a vessel as it goes through grey or white matter. The spaces have signal intensity similar to CSF on all sequences. Because they follow the course of penetrating vessels, they appear linear when imaged parallel to the course of the vessel, and round or ovoid, with a diameter generally smaller than 3 mm, when imaged perpendicular to the course of the vessel.

Cerebral microbleed

Small (generally 2–5 mm in diameter, but sometimes up to 10 mm) areas of signal void with associated blooming seen on T2-weighted magnetic resonance image (MRI) or other sequences that are sensitive to susceptibility effects.

Brain atrophy

A lower brain volume that is not related to a specific macroscopic focal injury such as trauma or infarction. Thus, infarction is not included in this measure unless explicitly stated.

Because CSVD affects small arteries, arterioles, venules, and capillaries in the brain which are too small to be detected by conventional neuroimaging, only T2-weighted MRI can quantify some of the resulting lesions such as white matter hyperintensities[68] (WMH), which is the most prevalent marker[67], comprehending a typical regional distribution both in the deep and periventricular white matter of the brain[69].

1.4. Periodontal Disease and Cardiovascular Disease

In the last decades, the possible association between periodontitis and cardiovascular disease has received much attention and several studies[70,71,72] have supported the existence of a relationship between periodontitis and an increased risk of CV disease. Periodontal disease has been assumed as a possible factor implicated in the etiopathogenesis of atherosclerosis[71,73,74,75] with both disorders presenting some common risk factors such as smoking, age and diabetes mellitus[71].

Recently, periodontal disease has been associated with CV disease, as periodontal bacteria have been found in several components of the CV system such as human cardiac tissue, pericardial fluids, heart valves and atherosclerotic lesions[76,77,78]. Thus, periodontal disease and consequent entry of bacterial products into the blood stream, conditioning the induction and maintenance of a chronic inflammatory state, has been associated with the onset and progression of CV disease[79,80,81,82].

Latest evidence seems to suggest that chronic oral infections such as periodontitis contribute to CSVD progression[83]. In fact, periodontitis risk factors intersect with those for CSVD and include aging, smoking, obesity, poor oral hygiene and diabetes mellitus[84,85,86,87]. Recent studies[88,89] found an association between early tooth loss and periodontal probing depth with presence and extension of WMH.

1.5. Inflammation and Cardiovascular Disease

Inflammation is also associated with CV disease and neutrophil – lymphocyte ratio (NLR) has been used as a marker of such disorder[90,91] as it is understood that neutrophils and lymphocytes are critical players both in inflammation and immune responses. NLR reflects the balance between the innate and adaptive immune mechanisms, and once elevated, pro-inflammatory cytokines may be increased too[92,93]. Thus, it can be used as a biomarker of the inflammatory response to chronic periodontitis as it is a simple and low-cost laboratory test that evidences the relation between periodontal disease and systemic conditions[94]. Previous studies[95,96] reported an NLR increase in patients with periodontitis and other inflammatory diseases, and a subsequent decrease with periodontal treatment[97].

1.6. Hypertension and Cardiovascular Disease

Hypertension is an inflammatory disease[18] which is the strongest indicator of CV outcome while evaluated within 24 hours monitoring, particularly during night-time period[98,99]. High blood pressure (BP) values starting around 115 mmHg of systolic BP and hypertension are recognized major risk factors for CV diseases contributing towards the high rate of coronary disease and stoke events[100]. Several international guidelines have defined hypertension based on office blood pressure (OBP) values, but ambulatory blood pressure (ABP) and home blood pressure are becoming more accepted as valuable tools to improve the characterization of the hypertension status [100,101]. ABP is a more reliable predictor of target organ damage and cardiovascular

morbimortality than OBP [102,103].

1.7. Arterial Stiffness and Cardiovascular Disease

Arterial stiffness, through its surrogate pulse wave velocity (PWV), has been proven to be an independent predictor of cardiovascular risk[103,104,105,106]. Peripheral BP, that is, the commonly used brachial BP, does not always appear as a reproducible surrogate of ambulatory pressure. All BP measurements with ABP, and OBP are then predictors of cardiovascular risk[103,104,105,106,107].

1.8. Salt Intake and Cardiovascular Disease

Also, high salt intake is a major factor that increases blood pressure being, therefore, responsible for strokes and heart attacks that involve proinflammatory mechanisms[108,109].

High salt intake is related to the increasing prevalence of hypertension and of cardiovascular risk[110,111]. The recommendations with respect to daily salt intake in adults should not exceed 5-6 g/day[100] based on its favourable effects on BP and on the presumable benefits in terms of reduction of CV morbidity and mortality. In the United States of America, it has been advised that daily salt intake limits for hypertensive patients should stay below 3.8 g[112]. Increased salt intake is associated with increased BP [110,111], whereas reducing sodium consumption decreases BP[111,113]. Medical scientific guidelines[100,114] have recommended a general reduction of dietary salt intake to less than 6 g/day. The association between BP and stroke is well established[115]. High salt intake has been linked to hypertension but also to stroke morbidity and mortality[116,117]. The relationship between salt consumption and CV disease has classically been considered as resulting from the influence of salt on BP. However, epidemiological[118,119], demographic[120] and animal[121] studies have provided evidence that salt intake may have an adverse effect on stroke mortality beyond and independent of BP. Portugal has a considerably high cerebrovascular mortality compared with other western countries[122,123]. Recently, in a representative sample of

the Portuguese adult population[124] it was shown that its mean salt intake was almost double the WHO recommendations.

2. OBJECTIVES

2. OBJECTIVES

The objective of this study was to investigate the association between periodontal disease indicators, CV disease risk factors and, more specifically, cerebral small vessel disease (CSVD).

Pico Question

In a population of adult hypertensive patients without previous cardiovascular or cerebrovascular events **(P)** is there an association between periodontal disease and cerebral small vessel disease **(O)** considering periodontal clinical parameters, cardiovascular risk factors and brain white matter hyperintensities **(C)**?

3. MATERIALS AND METHODS

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3.1. Subjects

A cross-sectional observational study was conducted in the Blood Pressure Unit of Hospital Pedro Hispano, Matosinhos, Portugal, which is an Excellence Center of the European Society of Hypertension. The local Hospital Ethical Committee approved the study protocol Nº 082/CE/JAS (appendix I), which was carried out in accordance with the Declaration of Helsinki. All subjects followed the routine clinical procedures, and a written informed consent was obtained (appendix II). Between 2016 and 2022, a convenience sample of forty-three Caucasian patients were included in this study, aged between 38 and 82 years and admitted to the Blood Pressure Unit of Hospital Pedro Hispano, Matosinhos, Portugal, without previous cardiovascular or cerebrovascular events. Participants demographic data were collected either by questionnaire in the first appointment or from existent clinical files: age, gender, height and weight, family history of cardiovascular risk and adverse outcomes, and calculated body mass index (BMI). Clinical data were recorded at baseline and included glycated hemoglobin (HbA1C), fasting plasma glucose (FPG), serum creatinine (estimated glomerular function according to Modification of Diet in Renal Study Equation), cholesterol (Total, HDL and LDL), triglycerides, ionogram, uric acid and 24h urinary sodium and potassium excretion controlled urinary creatinine level for evaluation of daily salt and potassium intake as previously described[125,126]. Participants were also examined regarding their chronic pharmacological therapies and habitual dietary and physical activity habits.

Diabetes mellitus was defined by two FPG ≥ 126mg/dl, 2h post-load plasma glucose ≥ 200mg/dl, HbA1C ≥ 6,5%, use of antidiabetic agents or personal history of diabetes [127,128]. Patients´ exclusion criteria were significant inflammatory disease, severe brain pathology (dementia by clinical criteria, brain tumor, traumatic brain injury, previous cerebral infection or neurodegenerative disease), previous cardiovascular events, changes in their ongoing therapy in the last 3 months, pregnancy, tumors, critical

illness or a life expectancy under 3 months, contraindication for magnetic resonance imaging (MRI) and inability to collaborate or to give informed consent.

3.2. Pulse Wave Velocity

PWV as an indicator of target organ lesion (atherosclerosis) was calculated using a validated non-invasive automatic device (Complior Analyze®, Colson; Garges les Gonesse, France) rated as excellent for PWV by the Artery Society criteria[129] (Fig. 3). Measurements were based in two pulse flow waves recorded simultaneously obtained at the level of the right common carotid and right femoral arteries, as previously reported[130].

Figure 3. Complior®Analyse device used to access arterial stiffness.

3.3. Ambulatory Blood Pressure Monitoring

Twenty-four-hour ABP monitoring was carried out using Spacelabs 90207 and 90217 (Spacelabs®, Redmond, Washington, USA). Measurements were taken as reported in previous studies[130] and performed according to international guidelines[131] (Fig. 4).

Figure 4. Ambulatory blood pressure monitors (ABPM). On the left the Spacelabs 90207 and on the right the Spacelabs 90217.

In OBP monitoring recordings were measured using automatic sphygmomanometer OMRON models M4-I and 705-IT (OMRON® Healthcare, Hoofddorp, Netherlands) (Fig. 5).

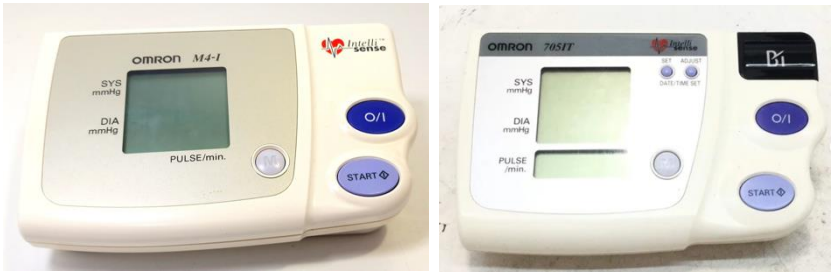

Figure 5. In office blood pressure monitors (OBPM). On the left the OMRON M4-I and on the right the OMRON 705-IT.

Patients were categorized in four circadian patterns according to nocturnal systolic blood pressure (SBP) fall, assessed as the continuous night-to-day ratio (NDR), transformed into percent reduction of daytime values: normal dippers (NDR (0.8; 0.9]), extreme dippers (NDR ≤ 0.8), reduced dippers (NDR (0.9; 1.0]) and reverse dippers (NDR > 1.0).

3.4. Global Cardiovascular Risk

Cardiovascular risk estimators are used to raise population awareness of cardiovascular diseases that cause a significant burden of morbidity and mortality[132]. The purpose of the Atherosclerotic Cardiovascular Disease (ASCVD) Risk Calculator is to estimate a patient's ASCVD risk in 10-year time at an initial visit to establish a reference point.

The information required to estimate ASCVD risk includes age, gender, race, total cholesterol, HDL cholesterol, systolic blood pressure, use of blood pressure lowering medication, diabetes status and smoking status. Estimates of 10-year risk for ASCVD are based on data from multiple community-based populations and are applicable to African American and non-Hispanic white men and women from 40 to 79 years of age.

The assessment of CV global risk, considering all the risk factors of an individual, enables at least, an orientation of the therapeutic measures. Several systems can be used to evaluate the CV risk of an individual.

The American Heart Association system is based in data from the Framingham Heart Study which is a cohort investigation on 5209 individuals that will also contribute to estimate the coronary disease risk[133]. Each algorithm of risk assessment includes different variables.

The standard variables, such as smoking, are quantified in different ways. The Framingham study calculates an estimated 10-year risk of CVD considering several parameters such as age, cholesterol, tobacco consumption, HDL cholesterol and systolic blood pressure[134]. The determination of CVD risk is of great importance since some clinical decisions rely on that estimate (e.g., timing of treatment start and therapeutic target). The absolute CV risk refers to the probability that an individual will experience a CV event within a given period considering the CV risk factors, target organ injury and previous CV event.

European guidelines for CVD prevention and arterial hypertension and dyslipidemia control[135] recommend using the Systematic Coronary Risk Evaluation (SCORE) system[136] to evaluate cardiovascular risk in countries with low risk rates and also in primary prevention (individuals who had never experienced a CV event). That system estimates the risk of an atherosclerotic event or death in 10-year time, considering the following risk factors: sex, age, smoking, systolic BP and total cholesterol level.

Risk calculation index using SCORE system, have been recently updated and published (SCORE 2)[136,137,138]. Several changes were made: the risk of a CV event is now added to the determination of the risk of CV mortality; the analysis was based in 45 cohort studies carried out in 43 countries, including 677.684 individuals and 30.121 CV events. Sex, age, smoking status and systolic BP were the variables used to estimate the risk, while the new index considers cholesterol instead of HDL. Diabetes mellitus is not included as it is considered a high-risk condition "*per se*".

Risk prediction models take in account the incidence of CV events in a certain country. Thus, four geographic risk regions were defined based on country specific CV events: low, moderate, high and very high risk.

For people aged between 70 and 89 years old, a risk adjusted model was developed (SCORE Older Persons) since risk of CV disease increases with age.

Some clinical conditions (level 3 high BP, hypercholesterolemia with LDL-c > 190 mg/dL, diabetes mellitus (DM), target organ injury, stage 3 or higher chronic kidney disease and established CVD) raise the risk level to high or very high. Relative risk and vascular age can be calculated in young adults with considerable increase in CV risk factors.

Vascular age table from SCORE gives information about the absolute risk and vascular age. Vascular age and relative risk can be estimated at any age and are clinically useful for non-high-risk individuals. High BP is one of the most important CV risk factors in what concerns to CV events. Primary high BP includes most of hypertensive population (90%) resulting from the complex interaction between genetic background, natural ageing process and several environmental factors[139,140,141].

Both genetic and surrounding elements operate through alterations in CV regulation mechanisms, causing an increase in vascular resistance, which is the responsible for BP elevation in almost hypertensive patients[142]. Nevertheless, high BP is also frequently associated to other CV risk factors such as dyslipidemia, glucose intolerance and type 2 DM, which will further increase CV risk[143,144].

In hypertensive patients, the coexistence of several risk factors (environmental, lifestyle, hypertension mediated organ damage, certain categories of chronic kidney disease and CVD) will influence CV risk. Specific risk factors are associated to women such as hypertension disorders in pregnancy and early menopause. Target organ injury is an important intermediate stage in the continuum of CVD between CV risk factors and CVD clinically manifest or advanced chronic kidney disease. Target organ injury (ventricular hypertrophy, aortic stiffness, albuminuria, kidney dysfunction, etc...) is an important determinant of global CV risk[145] which is usually increased in the presence of such condition[146]. DM is considered an independent factor that influences CV risk factors, regardless the presence of target organ injury, CVD or chronic kidney disease. The estimate of total CV risk is recommended in all hypertensive patients. Computerized methods were developed to estimate total CV

risk, i.e., the probability of developing a CV event usually in 10-year time. Many classification systems are based on the Framingham study but considering fatal and non-fatal cardiovascular event[147]. Framingham risk score can be applied to some European populations[148] but requires recalibration[149,150] due to geographic differences in the incidence of coronary events and stroke between European and north-American populations.

3.5. Periodontal Assessment

Information on periodontal condition was acquired by the same previously calibrated operator in all present teeth (six locations per tooth: mesial, central and distal from vestibular and palatal/lingual aspects) using a pressure-controlled manual periodontal probe (Hawe Click-Probe® No. 1391, KerrHawe® SA, Bioggio, Switzerland) with a unique click-system occurring always at a pression of ca. 20-25g (0.2-0.25N) which guaranteed reproducible and precise periodontal assessment (Fig. 6). A first assessment was obtained and two hours later a new one was registered. If differences between these two evaluations were found, a third measurement was performed and the mean between the three was considered.

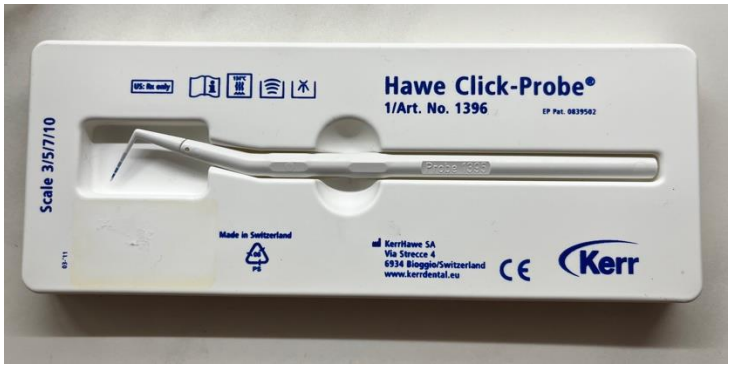

Figure 6. Pressure-controlled manual periodontal probe used to determine probing depth.

Clinical measurements were then inserted on a periodontal chart (www.perio-tools.com, Department of Periodontology, School of Dental Medicine, University of Bern, Switzerland) and mean probing depth (MPD), mean attachment level (MAL) and bleeding on probing (BOP) were obtained for each patient (Fig. 7)

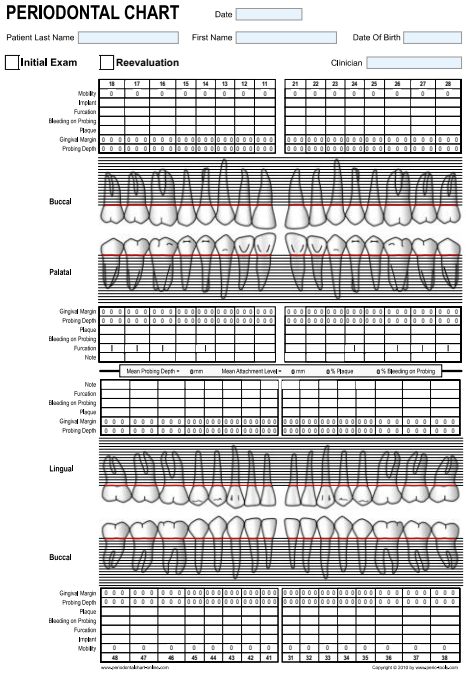

Figure 7. Periodontal chart used to collect patients´ periodontal condition.

To quantify the inflammatory burden posed by periodontitis and its risk factor for other diseases, periodontal epithelial surface area (PESA) and periodontal inflamed surface area (PISA) were also calculated[151] (Fig. 8).

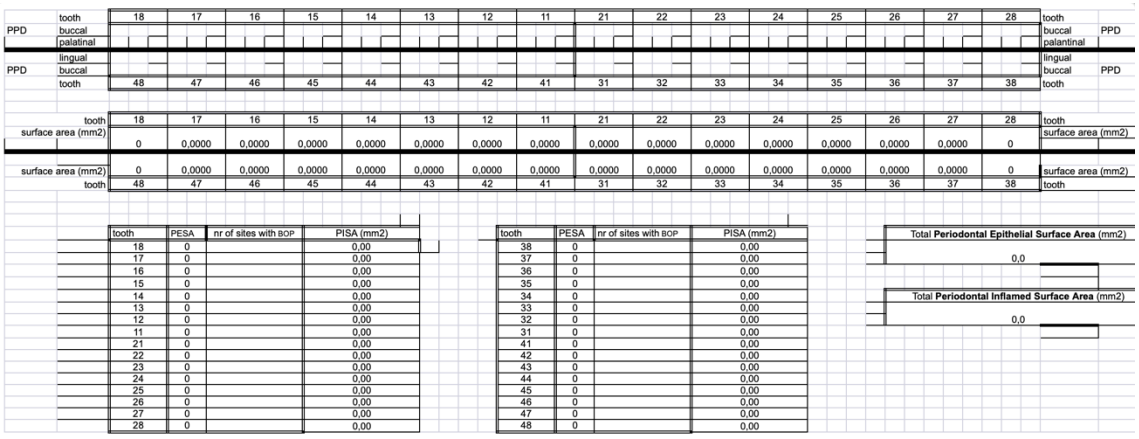

Figure 8. Freely downloadable spreadsheet used to calculate the PISA (https://www.parsprototo.info/).

Periodontal probing depth (PD) is the distance from the gingival margin to the apical portion of the gingival pocket and has been used as the main indicator for the presence of periodontal inflammation. In our study it was measured on six locations around each tooth: mesial, central and distal from vestibular and palatal/lingual aspects in both arches. In healthy subjects the depth of the gingival sulcus is 3 mm. However, in pathological conditions, the gingival sulcus is deeper, and a periodontal pocket is present. There are two types of pockets: supracrestal/suprabony pockets (when the inferior part of the sulcus is coronal to the alveolar crest) and subcrestal/infrabony pockets (when the inferior part of the sulcus is apical to the alveolar crest)[152,153]. The Community Periodontal Index for Treatment Needs (CPITN) for periodontal pocket depth was used and considered the following criteria to classify periodontitis regarding its severity: PD 0-3 mm: no/mild periodontitis; at least one pocket ≥4 mm and <6 mm: moderate periodontitis and with at least one pocket ≥6 mm as severe periodontitis[154].

3.7. Clinical Attachment Level

The clinical attachment level (CAL) is a parameter which indicates the loss of periodontal support around a tooth being an indicator of periodontitis severity[155,156] and concerns to the distance from the cemento-enamel junction (CEJ) to the bottom of the gingival sulcus.

The onset of an inflammatory condition in the connective tissue of the gingival sulcus causes the destruction of the gingival collagen fibers. In the apical area to junctional epithelium (JE), the collagen fibers degenerate and the local is filled with more inflammatory cells that migrate along the root to the apical area, while the coronal area detaches from the root surface, causing loss of attachment[157].

3.8. Bleeding on probing

Bleeding on probing (BOP) refers to the number of bleeding sites relatively to the number of probed sites and is used as one of the key diagnostic parameters for periodontal disease. In this study BOP was categorized as a dichotomous variable[158]. The American Academy of Periodontology / European Federation of Periodontology (AAP / EFP) consensus on the updated classification for periodontal and peri-implant diseases and conditions, considered the threshold for the diagnosis of stable periodontitis as well as gingivitis the existence of 10% of bleeding sites[35].

3.9. PESA/PISA

PESA precisely quantifies the total surface area of pocket epithelium. On the other hand, PISA refers to the area of PESA that is affected by BOP, and its value, in square millimeters (mm^2), represents de degree of periodontal inflammation and as such quantifies the systemic inflammatory burden which can be used to distinguish subjects regarding its periodontal inflammatory condition. It is calculated through the association of clinical parameters of periodontal diagnosis such as BOP and PD, CAL and gingival recession[151] and it has a relationship with parameters of vascular health and low-grade inflammatory systemic markers[159,160].
In this study both PESA and PISA were estimated using the average surface area of each tooth using a freely available Excel spreadsheet as previously described[151].

3.10. MRI

WMH volumes normalized by intracranial volume were derived from the T2-weighted fluid-attenuated inversion recovery (FLAIR) sequences collected in the sagittal plane. These FLAIR sequences are much more sensitive to reveal such chronic microvascular damage as hyperintense white matter regions.

Voxels resolution was 1 x 1 x 1, slices = 256, FOV = 256 mm, TR = 5000 ms, TE = 336 ms, TI = 1800 ms. Briefly, WMH masks were created using the Lesion Segmentation Algorithm (LPA, 1) from the Lesion Segmentation Toolbox for SPM12 in MATLAB R2018a. Following an initial segmentation of the FLAIR image, probability maps were binarized using AFNI (2,3, v21.0.15) command 3dcalc. Resulting segmentations were quality-checked for sufficient accuracy and volumes were calculated using Freesurfer (v7.1) command mri_segstats. Additional signs of CSVD were evaluated by an experienced vascular Neurologist to further characterize the CSVD in the patient cohort. Enlarged perivascular spaces (PVS) were defined as small (<0.3mm) punctate or linear (if perpendicular or longitudinal to the plane of the scan, respectively) hyperintensities on T2 images in the basal ganglia (BG) and centrum semiovale (CS)[161]. The PVS burden was then stratified into three groups: <11, 11–20 and >20[162]. Lacunes were defined as rounded or ovoid lesions >3mm and <20mm in diameter in the BG, internal capsule, CS or brainstem, with cerebrospinal fluid (CSF) density on T2 images[67]. Cerebral microbleeds were defined as round, hypodense lesions <10mm on susceptibility weighted imaging collected in the axial plane (slice thickness 2 mm, slices = 256, FOV = 230mm, TR = 49ms, TE = 40ms), according to the guidelines[163].

4. STATISTICAL ANALYSIS

4. STATISTICAL ANALYSIS

Most of the continuous variables assumed a non-normal distribution. After visual analysis and the Kolmogorov–Smirnov test, only age, 24h urinary sodium, daytime/night-time/24h pulse rate, in office SD DBP, night-time SD DBP, daytime DBP, and eGFR presented a normal distribution ($P > 0.05$).

To analyse the association between PD and clinical variables, significance level (α) of 0.05 was considered and Pearson's chi-square and Mann–Whitney rank sum tests were applied. We then performed generalized linear regression analysis (adjusted for significant clinical variables in univariate analysis and respective BP mean). Spearman's correlation coefficients (Rs) were calculated for the relationship between all variables and PD variables after adjustment. Analyses were performed using the SPSS statistical package (IBM SPSS statistics version 28.0.1.0, IBM Japan, Tokyo, Japan). All reported p-values were considered statistically significant if less than 0.05.

5. RESULTS

5. RESULTS

This study included 43 hypertensive patients, aged between 38 and 82 years (mean 63± 9), 39 with their natural dentition (91%), 18 (42%) female and 21 (50%) diabetics, all without previous CV events. All patients were eligible and agreed to undergo cerebral MRI with Siemens Aera 1.5T (Siemens Healthineers AG®, Erlangen, Germany) (Fig. 9) of which data from 6 patients were excluded regarding lack of quality for evaluations.

Figure 9. Siemens MAGNETOM Aera 1,5T MRI scanner.

Table 9 shows the descriptive analysis of the study sample.

Variáveis	N	Mean	SD
Age (years)	43	63,0	9,4
BMI (Kg/m2)	43	29,0	5,2
MMSE	41	27,9	2,0
MoCA	41	21,7	4,1
HbA1C (%)	42	6,4	1,2
Salt intake (g/day)	43	12,1	3,9
Creatinine (mg/dl)	42	1,0	0,4
GFR (ml/min/1.73)	42	78,0	22,9
Uric acid (mg/dl)	42	6,6	1,9
Total Cholesterol (mg/dl)	42	183,4	43,2
HDL- Cholesterol (mg/dl)	41	45,8	11,8
LDL- Cholesterol (mg/dl)	41	103,9	37,1
Triglycerides (mg/dl)	42	166,0	93,4
Albuminuria (mg/24h)	37	57,1	77,5
SBP 24 h (mm Hg)	39	132,8	12,2
DBP 24 h (mm Hg)	39	78,0	8,2
SBP daytime (mm Hg)	39	138,3	13,6
DBP daytime (mm Hg)	39	82,0	9,4
SBP night-time (mm Hg)	39	120,4	12,0
DBP night-time (mm Hg)	39	68,9	6,9
Night-time SBP fall (%)	39	12,7	7,2
Neutrophil / leucocytes ratio	39	0,5	0,2
Neutrophil / lymphocytes ratio	39	2,63	1,12
PESA (mm2)	39	1163,7	1551,4
PISA (mm2)	39	82,7	106,9
MPD (mm)	39	2,5	0,6
MAL (mm)	39	2,8	0,8
BOP	39	6,4	8,4
Number of teeth	39	17,0	10,1
WMH	37	0,34	0,53
Global CV Risk calculator	42	31,5	15,9
Pulse Wave velocity (m/s)	42	11.3	2.6

Table 9. Descriptive analysis of the sample.

All patients were under anti-hypertensive drugs, 52% were under statins and diabetic patients were all under oral antidiabetic medication. Mean probing depth of the sample was 2,5mm; mean periodontal inflamed surface area was 82,7mm^2; mean attachment level was 2,8mm and mean bleeding on probing was 6,4%.

In average, the assessment of global cardiovascular risk was 32 % for the probability of a fatal or non-fatal CV event in the next 10 years. In general, biochemical parameters were only slightly above the upper limits of normality. Also concerning hypertension control, average data from 24-hour ambulatory blood pressure, either during daytime or night-time, were slightly above upper limits of normality. Mean PWV value was above normal limits meaning an increase of aortic stiffness. As shown in Table 10, obese subjects (BMI equal or above 30 Kg/m2) vs non-obese showed significantly worse PD parameters (MPD and MAL).

	PISA	MPD	MAL	BOP
Obese - BMI v=30Kg/m2 (n=23)		2.69 + 0.53	3.02 + 0.83	
Non-Obese - BMI v=30Kg/m2 (n=15)		2.16 + 0.62	2.30 + 0.68	
p =		0.046	0.015	
Female - (n=17)	128 + 111			
Male - (n=20)	45 + 71			
p=	0.025			
NLRatio - above median - 3.0 (n=18)	162 + 131			11.3 + 10.9
NLRatio - below median - 3.0 (n=18)	31 + 754			3.2 + 4.4
p=	0.006			0.035
Salt intake - above median - 11g/d (n=18)			3.76 + 0.913	
Salt intake - below median - 11g/d (n=18)			2.270 + 0.73	
p=			0.048	
SBP nighttime - above median - 118mmHg (n=18)		2.70 + 0.65	3.10 + 0.68	
SBP nighttime - below median - 118mmHg (n=18)		2.11 + 0.51	2.32 + 0.71	
p =		0.047	0.011	

Table 10. Comparison (with statistical significance) of PD values between subgroups of subjects according to BMI, gender and values above and below medians of neutrophil/ lymphocyte ratio (NLR), salt intake and nighttime systolic BP.

Female vs male subjects showed worse values of PISA; Neutrophil-lymphocyte ratio values above median vs values below median were associated with significant worse values of PISA and BOP; nighttime systolic BP values above median vs values below median were associated with worse values of MPD and MAL; daily salt intake values above median vs values below median were associated with higher values of MAL. No significant differences of PD parameters were found between diabetics vs nondiabetics, between age values and between educational levels. The influence of smoking habits was not analysed since only 4 subjects were actual smokers (n=1) or previous smokers (n=3). Table 11 shows the numeric values of significant linear correlations between PD parameters and CV risk markers.

N=39		BMI	Salt intake (g/day)	Nighttime SBP	Neutrophil/ lymphocytes ratio	Global CV Risk	PWV
PISA	r2	**,385***			**,511****		**,443***
	p=	0,022			0,003		0,021
MPD	r2	**,468****	**,403***	**,441***		**,409***	**,502****
	p=	0,005	0,016	0,013		0,023	0,008
MAL	r2	**,349***	**,375***	**,461****		**,495****	
	p=	0,040	0,027	0,009		0,005	
BOP	r2	**,441****	**,344***	**,435***	**,571****	**,389***	**,470***
	p=	0,008	0,031	0,014	0,002	0,021	0,013

Table 11. Linear correlations statistically significant between periodontal and cardiovascular risk markers.

Body mass index correlated significantly with all four PD markers. Nighttime systolic BP, daily salt intake and Global CV risk correlated significantly with MPD, MAL and BOP.

NLR correlated with PISA and BOP and PWV with PISA, MAL and BOP. Figures 10 and 11 represent the simple scatter plots between some of the variables that in Table 11 were shown to be significantly correlated.

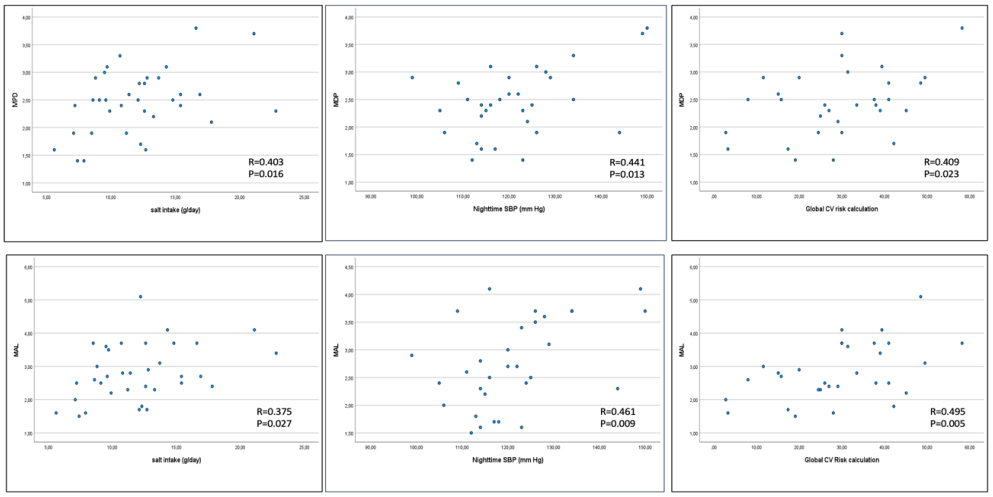

Figure 10. Scatter plot presenting the relationship between MAL and MPD with salt intake, nighttime SBP and global CV risk calculation.

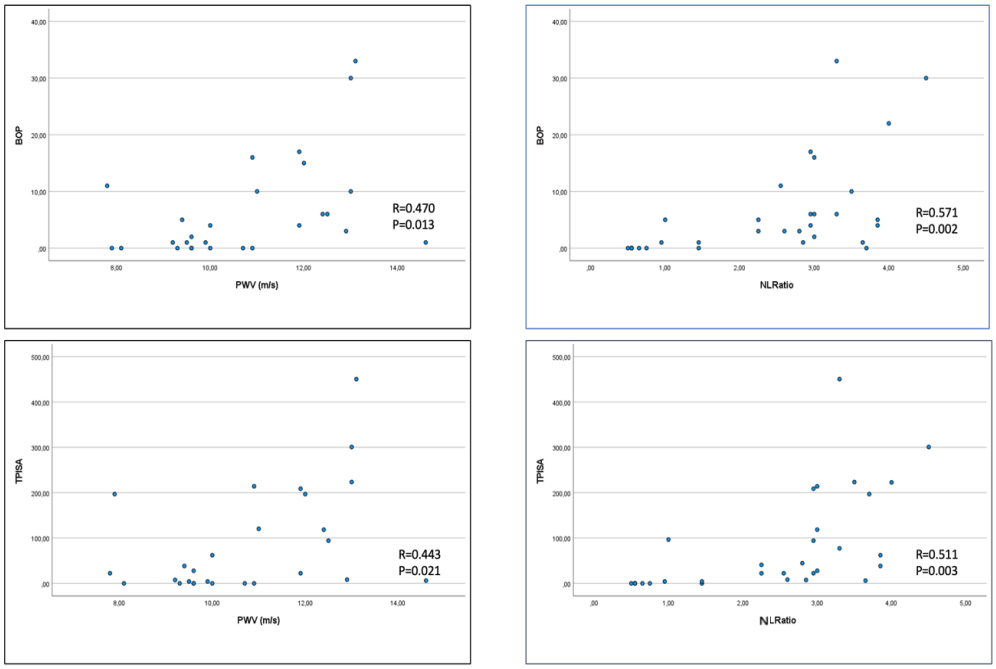

Figure 11. Scatter Plot presenting the relationship between TPISA and BOP with PWV and NLRatio.

Multivariate regression analysis presented in Table 12 shows that BMI, salt intake, NLR and nighttime systolic BP were independently and significantly correlated with BOP.

BOP	Coefficient padronized Beta	T	Sig.	CI 95,0% for B	
				Low	High
(Constant)		-4,480	0,000	-129,080	-46,661
BMI	0,324	2,154	0,045	0,013	1,033
SBP nighttime	0,329	2,270	0,036	0,021	0,555
Neutr/Lymph	0,326	2,317	0,032	1,202	24,533
Salt Intake	0,339	2,483	0,023	19,468	233,624

Table 12. Multiple regression analysis of cardiovascular risk markers and BOP.

		Gray matter	White matter
WMH	**Pearson correlation coefficient**	-,465	-,345
	Sig. (2 extremities)	,004	,039
	N	37	37

Table 13. Correlation between WMH and gray and white matter.

		WMH
TPISA	**Pearson correlation coeficient**	**-0,204**
	Sig. (2 extremities)	0,279
	N	37
MPD	**Pearson correlation coeficient**	**-0,145**
	Sig. (2 extremities)	0,445
	N	37
BOP	**Pearson correlation coeficient**	**-0,152**
	Sig. (2 extremities)	0,423
	N	37

Table 14. Correlation between TPISA, MPD, BOP and WMH (α=0.05).

		WMH
MAL	Pearson correlation coeficiente	0,015
	Sig. (2 extremities)	0,937
	N	37

Table 15. Correlation between MAL and WMH (α=0.05).

6. DISCUSSION

6. DISCUSSION

In the present study that enrolled a population of hypertensive patients without previous cardiovascular events, we found that periodontal disease severity was significantly associated with the severity of the calculated overall cardiovascular risk as a 10-year predictor of fatal and non-fatal cardiovascular events. Also, periodontal disease is associated with cardiovascular risk factors such as ambulatory blood pressure particularly with nighttime values, with target organ damage markers such as aortic stiffness (PWV), with markers of cardiovascular damage and inflammatory promoters such as high salt intake and with indicators of abnormal immune system homeostasis evaluated with circulating NLR. Moreover, multivariate regression analysis emphasized an independent direct relationship between these cardiovascular risk variables and bleeding on probing severity.

Multiple studies have proven the existence of a close association between periodontal disease and cardiovascular disease[70,71,73,74,75,76]. However, the question of causality is still unclear particularly because most of studies have used only surrogate markers/biomarkers as endpoints. The possible practical solution to this issue is not ethically feasible which limits the execution of studies involving untreated groups.

Recently, inflammation has been considered an important contributing factor for both periodontal and cardiovascular diseases[81,82,164,165]. Considering this, we also intended in this study to evaluate the relationship between periodontal disease and cardiovascular risk markers in which the contribution of the inflammation factor is well established, both in its pathophysiology and in its consequences. We found a clear association between periodontal markers and cardiovascular risk, globally considered. That is, our data confirm the great weight of evidence that patients with a higher probability of cardiovascular events have greater periodontal disease severity[71,72,73,74,75,81,166]. This, thereby predictable finding, was reinforced by the relationship found between the various markers of periodontal disease and the aortic stiffness evaluated by the pulse wave velocity. Since pulse wave velocity is a marker of target organ damage and an integrated indicator of cardiovascular disease and

atherosclerosis, the relationship found between pulse wave velocity and periodontal disease confirms the results of other authors and constitutes an internal validation of the present study. Periodontal disease has been considered as an inflammatory disease[81,82]. We found a significant and independent association between risk indicators for periodontal disease severity and the NLR. This ratio is calculated by dividing absolute neutrophil to absolute lymphocyte count. NLR is a simple index for assessing inflammation. Emerging evidence suggests that increased NLR is a potential marker for poor prognosis in multiple tumors, being associated with a higher risk of cardiovascular events and mortality rate[91,165,167]. Higher levels of peripheral leukocytes in periodontitis patients have been reported on several studies[97,168,169,170]. Furthermore, in one study including 27 patients with generalized aggressive periodontitis there was a decrease in the numbers of leukocytes after non-surgical periodontal therapy[20].

Another work, where the blood cell components in patients with localized and generalized periodontitis where studied, concluded that the number of peripheral WBC was higher with the increasing severity and extent of periodontitis[171]. An independent relationship between the severity of periodontal disease and the number of WBC was also found[172]. Furthermore, one study confirmed that patients with aggressive periodontitis had higher counting of peripheral WBC than controls. This same study also reported that the total numbers of leukocytes and neutrophils were positively associated with the severity and extent of periodontitis[95].

In our study, NLR values above median were associated with worse values of PISA and BOP. Thus, our data confirmed a clear association between periodontal disease with that (NLR) well established inflammatory status. Nevertheless, it should be mentioned that because NLR is increased in numerous systemic disorders, periodontitis patients might present neutrophil-lymphocyte ratio values that are representative of both conditions. Thus, if on one hand it is important to be aware of the potential confounding factors that may influence neutrophil-lymphocyte ratio value, on the other one these confounding factors may represent themselves the link between periodontal disease and other systemic inflammatory conditions. Considering that NLR is inex-

pensive and easy to obtain[94], it might be useful as an aid monitoring patients' periodontal conditions. We found a significant and strong independent association of high blood pressure values and periodontal disease indicators. This was particularly relevant for nighttime systolic blood pressure values which are, among the different ways of measuring blood pressure, those with a better performance for diagnosis of hypertension and with the highest predictive value for cardiovascular outcome and events while compared with blood pressure recorded in office, at home or during daytime periods[98,99,130]. Several studies have reviewed the available data on the relation between periodontitis and hypertension[70,80,173]. Nighttime blood pressure values were significantly correlated with periodontal parameters (MPD, MAL and BOP) and independently associated with bleeding on probing in multivariate analysis. Also, nighttime blood pressure values above median were associated with worse levels of attachment level.

Both periodontal disease and hypertension share a common inflammatory pattern[81,82,164]. Hypertension is a major risk factor for cardiovascular disease[131] being associated with increased systemic or local vascular inflammation and oxidative stress[164]. High blood pressure has an impact on several organs and tissues. In the vascular system, causes endothelial dysfunction, generalized atherosclerosis, arteriosclerotic stenosis, remodeling of small and large arteries and aortic aneurysm.

Regarding the heart, it can cause left ventricular hypertrophy, atrial fibrillation, coronary microangiopathy, coronary heart disease, and heart failure. In the brain, high blood pressure increases the risk of acute hypertensive encephalopathy, stroke, intracerebral hemorrhage, lacunar infarction, focal or diffuse white matter lesions, and vascular dementia. When affecting the kidney, it leads to albuminuria, proteinuria, reduced glomerular filtration rate, chronic renal insufficiency, and, ultimately, renal failure[174,175].

Severe periodontitis is strongly associated with hypertension[173] and some studies have claimed to observe a decline of blood pressure after periodontal disease therapy[176]. Thus, our most striking finding was the association of periodontal parameters with 24 h ambulatory blood pressure, and among the ambulatory blood pressure monitoring data just with nighttime blood pressure i.e., the blood pressure values that have

the best predictive weight for cardiovascular events[98,99,130].

In our study, high salt intake was significantly associated with probing depth, attachment level and bleeding on probing, and independently associated with the last one.

Also, salt intake values above median were associated with worse levels of attachment level. High salt intake is particularly common in our country, being around 10.8 g/day[126], almost twice the optimal recommended intake level[131]. As in previous studies[125,126,124], we measured daily salt intake by the most accurate method i.e., by determining the sodium excretion in at least two valid 24-hour urinary samples. In the present study, average salt intake was even higher than the mean daily salt intake of the Portuguese population[126]. High salt intake has been implicated not only on the blood pressure rise but also by promoting cardiovascular disease and cerebral damage through some well stablished pro-inflammatory mechanisms[109,125,177]. Salt can damage the endothelium through inflammatory mechanisms and also through oxidative stress since the administration of antioxidants has been shown to reverse that damage[178,179].

Studies show that a high salt intake impaired flow-mediated dilation and increased arterial stiffness[180,181]. On the other hand the effect of the reduction of salt intake causes reverse effects[182] and is shown to be independent of blood pressure[179,183,184].

Interestingly, we found a relationship between high salt intake and periodontitis. Inflammation appears to link periodontal disease and high salt intake, thereby being possible, although still speculative, that high salt intake may importantly contribute to the inflammatory reaction within the spectrum of periodontal disease.

Beyond the oral careless habits, factors contributing to periodontal disease include socioeconomic status, gender, education, diet, and smoking[185]. In the present study women had worse conditions of some periodontal markers (PISA and BOP) than men, but no differences were found on periodontal status between diabetics vs non diabetics and high vs low educational level. That may be due to the small numerical size of our population.

This study demonstrated a statistically significant inverse association between WMH and gray and white matter (Table 13).

This outcome was expected as WMH is considered to constitute the earliest damage of cerebral small vessel disease, mostly involving subcortical white matter[186].

Surprisingly, data analysis also revealed that lower values of TPISA, MPD and BOP were related with more WMH lesions (Table 14).

Better said, we didn't expect that an increase in WMH identified by MRI were associated with less periodontal inflamed areas, reduced pocket probing depths and lower bleeding on probing percentages. The explanation for these findings might be associated with the proper nature of the periodontal indicators that were used even though these results are the opposite of those previously described in other studies. Nevertheless, we should emphasize that those same studies with which we compared our results, employed different methods, susceptible of unfair outcomes.

When Minn et al[88] investigated the association between tooth loss and brain white matter changes, 650 patients were selected of which 438 underwent dental examination that by no means didn't characterize periodontally the patients enrolled in the study but, instead, assumed that the number of lost teeth reflected their periodontal condition. Moreover, they considered as control group patients who had lost 1 to 5 teeth, and only patients with 6 or more lost teeth as periodontally compromised, even though no periodontal examination was done. Basically, they presumed that the loss of 1 to 5 teeth was because of cosmetic problems, wisdom tooth pain or trauma, without the presence of any underlying periodontal disease[88]. Considering this approach, for example, a non-periodontal patient that had both maxillary and mandibular wisdom teeth removed and two other teeth extracted for orthodontic reasons, was considered periodontally compromised. Thus, this methodology arouses several doubts regarding the genuine periodontal condition of the patients participating the study. Nevertheless, and assuming that the lower the residual amount of supporting bone the higher the probability of tooth loss [187], then results obtained by Minn et al[88] are in line with those collected in our study.

Another critical difference concerning the methodology that was used, and with real impact in the results obtained, is related with the fact that brain white matter changes were diagnosed by computerized tomography (CT) rather than MRI, which we know has higher sensitivity and specificity for detecting pathological changes[67].

Regarding mean attachment level, results suggest a positive association with WMH, however not statistically significant (Table 15).

We believe that this lack of statistical significance is related with the reduced size of the sample used in our study. Nevertheless, we consider relevant that it is MAL, as a determinant of the accumulated periodontal destruction, that is associated with higher WMH, contrary to what happens with TPISA, MPD and BOP, all of which representing the periodontal condition "in the moment", and so not as representative as attachment level in what concerns to the cumulative nature of the brain white matter degeneration.

Also, Hada et al[89] studied the relationship between periodontal condition and white matter hyperintensities in an adult Japanese population. For such, 444 patients were enrolled in the research. Magnetic resonance image was used to identify brain white matter changes, and patients were classified in four different categories accordingly with presented brain lesions. Periodontal status of the patients involved in the study was obtained by measuring probing depth in 10 teeth per patient, regardless of the number of existing teeth. Therefore, whenever a patient presented at least one probing depth of ≥ 4mm was classified as moderate periodontitis. Severe periodontitis was designated if there was one or more probing depth ≥ 6mm. Results revealed a statistically significant association between white matter hyperintensities and probing depths ≥ 6mm. For probing depths ≥ 4mm there was no significance. Thus, the author concluded that severe periodontitis might influence the risk of developing white matter hyperintensities. Contrasting with what Hada et al[89] have found, our data analysis showed an inverse association, not statistically significant, between white matter and mean probing depth.

More precisely, higher number of WMH were associated with lower values of probing depths. We explain this outcome not only because of the small sample that was used, but also, and once again, because probing depth only describes the periodontal condition in the moment and so, it is not a trustworthy indicator of the cumulative nature of periodontitis and, consequently, of the resultant association with WMH.

Mayer et al[188],more recently, aimed to establish the association between periodontal disease and the microstructural brain alterations in the Hamburg City Health Study (HCHS) which was an ongoing, single-center, prospective, epidemiological cohort study with emphasis on imaging to improve the identification of individuals who are at risk for major chronic diseases and to improve early diagnosis and survival. Data from 2030 patients were included in the analysis. Certified study nurses determined participants' probing pocket depth, gingival recession and consequent attachment loss. No reference was given regarding intra and inter operator calibrations. All patients participating the study were submitted to 3-T magnetic resonance image. Once adjusted to gender and age, results showed that all periodontal indicators were significantly associated with more white matter hyperintensities, and, mainly, the more the attachment loss the higher the number of white matter lesions. On the other hand, and when the statistical model was adjusted to education and cardiovascular risk, only plaque index remained significantly associated with white matter hyperintensities, with attachment loss positively associated with white matter hyperintensities, but without statistical significance, literally as it was reported in our study. We must refer that, in this work designed by Mayer et al[188], the quantification of WMH was more precise, because the methodology selected permitted the identification of subtle brain microstructural changes, well before these produced visible vascular damage by conventional magnetic resonance image (1,5T). Thus, we can affirm that specifically in this study, there were identified brain white matter lesions that wouldn´t have been if standard MRI was used. By doing so, obviously, WMH were overvalued.

Chronic inflammation represents an important role in the development of cerebral small vessel disease, because of its nefarious effects over the healthy tissues. Once inflammatory mediators reach endothelial cells can cause the rupture of the brain blood barrier[189,190,191], being this the mechanism underneath the pathogenesis of the disease, since it facilitates the transit of harmful substances to brain tissues, causing vascular changes[192]. This is the reason why, plausibly, periodontitis, being one of the most common chronic infection of the oral cavity, assumes a key role in what concerns to the association with cerebral small vessel disease, considering the

chronicity and complexity inherent do this oral disease, along with the persistent inflammatory reaction transmitted to the teeth supportive tissues and consequent discharge of numerous inflammatory mediators, leading to endothelial dysfunction which plays a critical role in the pathogenesis of cerebral small vessel disease. The fact that periodontal treatment enhances endothelial function, strengths the linkage between these diseases[193] and, therefore, periodontal patients should have an increased risk for CSVD[194]. Due to the anatomic proximity between the mouth and the brain, bacteria such as Porphyromonas gingivalis (PG), which is found in gingivitis and periodontitis, can travel through blood stream and reach the cerebral tissue. Corroborating this theory, a study from Lee et al[195] showed that periodontal treatment and dental prophylaxis towards PG can prevent ischemic stroke. Also, studies have demonstrated an association between the presence of PG and an increased risk of ischemic stroke[196] and cardioembolic and thrombotic stroke[197].

7. CONCLUSIONS

7. CONCLUSIONS

This study not only confirms the association of periodontal disease and cardiovascular risk factors on a population of hypertensive patients without previous CV events, but also has evaluated the relationship of periodontal disease with pro-inflammatory parameters rarely studied in this context such as salt intake, neutrophil-lymphocyte ratio and ambulatory blood pressure monitoring. Also, white matter hyperintensities were correlated with a determinant of periodontal disease which is the more accurate indicator of the periodontal support around a tooth. Nevertheless, it remains unclear whether the relationship found between these inflammatory parameters, white matter hyperintensities and periodontal disease is purely circumstantial because of the simple coexistence of high prevalent phenomena. Thus, the question of a possible causal relationship between periodontal disease and cerebro-cardiovascular disease remains clearly unsolved.

8. FUTURE RESEARCH

8. FUTURE RESEARCH

This study has some limitations such as enrolling a small population within a narrow age limit and restricted to a single location. All participant patients were hypertensive, with high cardiovascular risk and submitted to several medications, so the generalization of the results to other populations is obviously limited. Since it is a cross-sectional observational study, we cannot affirm the causality between periodontal disease, cardiovascular risk and pro-inflammatory markers and white matter hyperintensities. Large prospective studies are therefore needed in order to enhance the state of current evidence regarding periodontal disease, brain lesions and cv risk markers.

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APPENDIX

APPENDIX

Appendix I - Ethics committee study approval

UNIDADE LOCAL DE SAÚDE DE MATOSINHOS HOSPITAL PEDRO HISPANO	INFORMAÇÃO	Nº 082/CE/JAS Data: 12-12-2014

Para: **Rui Silva (SEGIC)**
De: **Comissão de Ética**

Assunto: **Pedido de autorização para a realização de um estudo intitulado "Doença de pequenos vasos cerebral: novos métodos para avaliação de risco e prevenção"**

INFORMAÇÃO

Exmos. Senhores,

A Comissão de Ética, analisou na sua reunião de 12 de Dezembro de 2014, o pedido de autorização para a realização de um estudo intitulado "Doença de pequenos vasos cerebral: novos métodos para avaliação de risco e prevenção", proponente Dra. Ana Monteiro da Unidade de Investigação Cardiovascular, a realizar no âmbito da tese de doutoramento em Neurociências Clínicas, Neuropsiquiatria e Saúde Mental da Faculdade de Medicina da Universidade do Porto.

Decidido nada opor à realização deste estudo.

Com os melhores cumprimentos

Dr. José Alberto Silva
Presidente da Comissão de Ética
da ULSM Matosinhos

Dr. José Alberto Silva
(Presidente da Comissão de Ética da U. L. S. – Matosinhos)

001_01_INF_1260 Unidade Local de Saúde de Matosinhos

Appendix I - Ethics committee study approval (cont.)

Exmo. Sr. Diretor da Unidade de Hipertensão e Risco Cardiovascular do Hospital Pedro Hispano (também Presidente da Comissão de Ética)

Sr. Dr. José Alberto Silva

Tiago Saturnino Amaral Pinto Ribeiro, médico dentista (OMD 5065), no âmbito da elaboração da sua tese de doutoramento a apresentar à Faculdade de Medicina Dentária da Universidade do Porto, intitulada "Estudo da associação entre a doença dos pequenos vasos cerebrais e a doença periodontal", e integrada no estudo intitulado "Doença dos pequenos vasos cerebral: novos métodos para a avaliação de risco e prevenção", cuja investigadora principal é a Dra. Ana Monteiro, e que recebeu parecer favorável à sua realização pela Comissão de Ética em 12-12-2014 (nº 082/CE/JAS), vem por este meio, e na sequência do mesmo, solicitar a V. Ex. autorização para poder avaliar o estado de saúde periodontal dos mesmos doentes incluídos no estudo previamente aprovado, e que são seguidos na consulta de hipertensão do Hospital Pedro Hispano. Esta avaliação, não invasiva, demorará cerca de 15 minutos por doente.

Desde já grato pela atenção dispensada,

Respeitosos cumprimentos,

Tiago Pinto Ribeiro

DR. JOSÉ ALBERTO SILVA
Medicina Interna-CS
Nº Mec. 56 - C.P. 17845

Porto, 15 de Abril de 2016

Appendix II - Informed Consent

CONSENTIMENTO INFORMADO, ESCLARECIDO E LIVRE, PARA INVESTIGAÇÃO CLÍNICA

Título do Estudo:
Em português: Doença de pequenos vasos cerebral: novos métodos para avaliação de risco e abordagem preventiva

Em Inglês: Cerebral small vessel disease: new tools for preclinical risk assessment and preventive management

Centros envolvidos:
Hospital Pedro Hispano (Unidade Local de Saúde de Matosinhos, EPE)
Grupo de Investigação Cerebrovascular, Unidade de Investigação e Desenvolvimento Cardiovascular da Faculdade de Medicina da Universidade do Porto (FMUP)
Unidade de Neurologia e Neurocirurgia do Departamento de Neurociências Clínicas e Saúde Mental da FMUP

Investigador Principal:
Ana Maria Gonçalves Monteiro

PARTE A - INFORMAÇÃO AOS PARTICIPANTES

Introdução
O meu nome é Ana Monteiro e sou médica do Serviço de Neurologia do Centro Hospitalar de São João e investigadora do Grupo de Investigação Cerebrovascular da Faculdade de Medicina da Universidade do Porto. Gostaria por este meio de o informar e convidar a participar deste projeto de investigação.
Este documento irá descrever o estudo, por favor leia-o atentamente e se não se sentir completamente esclarecido(a) sinta-se à vontade para colocar qualquer questão ao investigador(a) presente. Não tem de decidir se quer participar neste momento. Antes de decidir participar pode falar com quem desejar acerca deste estudo. Se restarem quaisquer dúvidas após a entrevista, poderá esclarecê-las contactando o investigador(a) através dos contactos disponibilizados no final deste documento.

Objetivos do estudo
A doença de pequenos vasos (DPV) cerebral tem um enorme peso na saúde pública mundial, sendo mais expressiva ainda em Portugal. Corresponde à doença dos mais pequenos vasos sanguíneos cerebrais, sendo responsável pela maioria dos acidentes vasculares cerebrais (AVC) hemorrágicos e por 25% dos AVC isquémicos. Tem efeitos catastróficos na capacidade mental, sendo a segunda maior causa de demência e contribuindo para o surgimento e agravamento da doença de Alzheimer, e é a principal causa de incapacidade nos idosos. Assim, é imperioso criar meios para a prevenção e tratamento desta entidade. No entanto, os fatores e os mecanismos que contribuem para o seu desenvolvimento são ainda pouco conhecidos, tornando difícil uma correta atuação médica. O presente projeto pretende aprofundar o

conhecimento sobre a DPV cerebral e avaliar novos exames, seguros e fáceis de usar, para diagnosticar esta doença mais cedo, antes das suas consequências surgirem.

Seleção dos participantes

Foi selecionado(a) para participar neste projeto por ter hipertensão arterial, com ou sem diabetes mellitus, e não ter ainda manifestações cerebrais destas doenças.

Procedimentos do estudo

Este estudo envolve a avaliação na sua consulta habitual de hipertensão arterial, bem como a realização de uma série de exames. Para além da observação clínica e da realização do exame objetivo, estão previstos os seguintes procedimentos:

- Colheita de uma amostra de sangue periférico e de urina (os já rotineiramente pedidos anualmente na sua consulta para estudo da hipertensão arterial e diabetes mellitus);
- Avaliação com MAPA de 24h, eletrocardiograma (ECG) e medicação da rigidez da aorta;
- Realização de estudos de neurossonologia: ecoDoppler dos vasos do pescoço e transcranianos, com estudos de vasorreatividade cerebral, na unidade de Investigação Cerebrovascular da FMUP;
- Observação por oftalmologia no Centro Hospitalar de São João, com avaliação por Tomografia de Coerência Óptica (OCT), uma técnica não invasiva, facilmente reprodutível e de rápida execução, que permite avaliar a espessura das camadas de fibras nervosas e vasculares da retina;
- Realização de ressonância (RM) cerebral, uma técnica de imagem cerebral que não utiliza radiação;
- Realização de avaliação neuropsicológica, com uma bateria de testes para avaliar a capacidade cognitiva, a realizar na FMUP.

Para a maioria dos procedimentos, não estão previstos riscos ou desconforto para os participantes. A única exceção é a colheita de sangue periférico por punção venosa. No entanto, esta colheita não implica uma picada extra e será realizada no contexto das suas análises de rotina.

Duração do estudo

O estudo tem uma duração de 3 anos. A sua participação estará terminada no máximo dentro de 3 meses após a sua entrada no estudo. Idealmente, serão realizadas avaliações no seu hospital no dia da sua consulta (MAPA, ECG, medição da rigidez da aorta, colheita de sangue e urina) e na FMUP no menor número de dias possível, realizando mais de um exame no mesmo dia (RM cerebral, estudos de neurossonologia, de oftalmologia e avaliação neuropsicológica).

Vantagens de participar

Ao escolher participar neste estudo estará a contribuir para aumentar o conhecimento acerca da doença de pequenos vasos cerebrais e para o desenvolvimento de novas formas de avaliar os doentes em risco, de forma a podermos atuar mais precocemente e com maior eficácia, prevenindo o surgimento da doença. Irá também realizar uma série de exames que serão úteis para a orientação do seu tratamento.

Custos e reembolsos

A sua participação neste estudo envolve encargos financeiros para além dos necessários à sua avaliação clínica habitual, nomeadamente com a realização dos exames não invasivos. O custo desses exames não será creditado a si ou ao HPH, mas sim serão pagos por fontes de financiamento externo, obtidas para este estudo, ainda a determinar. Será incluído, ainda, o custo do seu deslocamento às instituições, quando este ocorra para além da sua avaliação clínica habitual. A sua participação neste estudo é totalmente voluntária, não receberá qualquer compensação monetária por participar no estudo.

Participação voluntária

A sua participação neste estudo é voluntária, se decidir não participar no estudo em nada será prejudicado e manterá o seguimento regular previsto na Consulta de Hipertensão Arterial do HPH. Se decidir participar, pode abandonar o estudo em qualquer altura, sem qualquer detrimento para a continuação do seu seguimento/tratamento. Para tal, basta comunicar a sua decisão (pessoalmente ou através dos contactos abaixo). De forma alguma a sua decisão de participação ou não neste estudo terá impacto na forma e qualidade da assistência médica e na orientação do diagnóstico e terapêutica de acordo com as normas médico-científicas atuais.

Confidencialidade

Os registos pessoais obtidos durante a sua participação neste estudo, assim como os respetivos dados de saúde e resultado dos exames são estritamente confidenciais, sendo o seu acesso restrito à equipa de investigação. A informação será codificada e utilizada apenas neste estudo. Os seus dados poderão ser usados em futuras publicações, respeitando as regras de confidencialidade. Não serão nunca publicados dados individuais ou identificativos da sua pessoa. Os dados serão apenas conservados durante o tempo necessário à publicação dos resultados. Se o pretender, todos os seus dados pessoais e informação recolhida podem ser acedidos, corrigidos ou apagados.

Este estudo foi submetido e mereceu um parecer favorável da Comissão de Ética do Hospital Pedro Hispano e da Comissão Nacional de Proteção de Dados.

Questões sobre o estudo

Antes de assinar este consentimento, deve colocar as todas as questões ou dúvidas que pretender. A equipa do estudo está disponível para responder a questões antes, durante e após o estudo.

Poderá contactar a coordenadora deste estudo, a médica Ana Monteiro, utilizando os seguintes contactos:

Tel: 916216952 ou 963926541

E-mail: ana.mg.monteiro@gmail.com

PARTE B - CERTIFICADO DO CONSENTIMENTO

Título do estudo: Doença de pequenos vasos cerebral: novos métodos para avaliação de risco e prevenção

Confirmo que expliquei ao participante/representante legal, de forma adequada e compreensível, a investigação referida, os benefícios e riscos associados à sua realização. O participante afirmou ter compreendido a minha explicação.

O investigador que obteve o consentimento: _____
Assinatura: _____
Data: _____/_____/_____

Declaro que li e compreendi o conteúdo deste documento, bem como as informações verbais que me foram facultadas pelo(a) investigador(a). Declaro que o objetivo e procedimentos do estudo me foram devidamente explicados, bem como os benéficos, riscos e eventual desconforto. Solicitei todas as informações de que necessitei, sabendo que o esclarecimento é fundamental para uma boa decisão. Declaro que aceito que os meus dados sejam utilizados para os objetivos descritos no estudo, sabendo que os mesmos serão tratados confidencialmente, omitindo o meu nome e protegendo a minha imagem. Fui informado da possibilidade de livremente recusar ou abandonar a qualquer momento a participação no estudo, sem que isso tenha qualquer prejuízo na assistência que me é prestada. Declaro não ter sido incluído em nenhum outro projeto de investigação nos últimos três meses.
Assim, concordo com a participação neste estudo, de acordo com os esclarecimentos que me foram prestados, como consta neste documento, do qual me foi entregue uma cópia.

Nome do participante: _____
Assinatura: _____
Data: _____/_____/_____

Se não for o doente a assinar:
Nome: _____
Assinatura: _____:_____
BI/CC nº:_____Data/validade: _____/_____/_____
Grau de parentesco ou tipo de representação:_____

Este documento é composto por 5 páginas e feito em duplicado:
uma via para o investigador (pág 5) e uma via para a pessoa que consente (pág 1-4)

FACULDADE DE **MEDICINA DENTÁRIA**