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OSTEOPOROSIS AND JAWBONES: DENTAL PANORAMIC RADIOGRAPHS TO SCREEN FOR POST-MENOPAUSAL OSTEOPOROSIS AND CORRELATION BETWEEN PERIODONTAL STATUS AND BMD

OSTEOPOROSI E OSSA MASCELLARI: UTILIZZO DI RADIOGRAFIE PANORAMICHE PER ESEGUIRE SCREENING DI OSTEOPOROSI POSTMENOPAUSALE E CORRELAZIONE TRA STATUS PARODONTALE E BMD

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Ai miei genitori,

Mario e Elena

"Genera più spesso confidenza l'ignoranza di quanto non faccia la conoscenza"

- Charles Robert Darwin -

ABSTRACT

Osteoporosis is the most common chronic bone disease that may interfere with bone metabolism. This PhD thesis aimed to investigate the effect of osteoporosis on the jawbones, in particular the relationship between the systemic bone density and the jawbone conditions.

Firstly, a literature review on the effect of osteoporosis on the jawbones and on the accuracy of panoramic morphometric indices to screen for reduced bone mineral density were presented. Despite the poor quality of the available studies, our data showed that a correlation between jawbone and skeletal density in osteoporotic patients might be expected. A second critical review on the correlation between osteoporosis and periodontal condition of the patients were done. Similarly to the aforementioned review, despite the quality of the papers, a correlation has been suggested.

Secondly, a clinical trial to compare BMD and jawbones condition in terms of bone density and periodontal status were presented. Seventy patients, that were done a Dxa scan to investigate the BMD in the previous year, were enrolled and a complete periodontal data collection as well as a standardized ortopantomography were done. T-scores from the Dxa scan were used to classify patients in healthy or osteoporotic and specific morphometric indices were measured on the OPG.

Finally, a statistical analysis was performed and a significant correlation was found between BMD and a combination of two indices: the qualitative “Klemetti index” and the quantitative “mandibular cortical width”. The accuracy of these indices was calculated and the combination showed a high predictive value in detecting healthy patients. The statistical analysis applied to the periodontal indices showed that pocket probing depth ≥ 5 mm and attachment level 3-4 mm were correlated to healthy condition. These controversial results may be related to the limitations of the study that include the reduced sample size and the recruitment centre.

RIASSUNTO

L'osteoporosi è la più comune patologia cronica che influenza il metabolismo osseo. Questa tesi di dottorato mira a investigare gli effetti dell'osteoporosi sulle ossa mascellari e in particolare la relazione che intercorre tra densità ossea sistemica e le condizioni del cavo orale.

Inizialmente, è stata effettuata una revisione della letteratura circa gli effetti dell'osteoporosi sulle ossa mascellari e riguardo all'accuratezza di specifici indici morfometrici per effettuare screening di ridotta densità ossea minerale. Nonostante la modesta qualità degli studi a disposizione, i dati dimostrano una correlazione tra densità ossea sistemica e mascellare nei pazienti osteoporotici. Allo stesso modo, è stata eseguita una seconda revisione critica sulla correlazione tra osteoporosi e malattia parodontale. Anche in questo caso una correlazione è stata suggerita.

Secondariamente, è stato eseguito un trial clinico per comparare BMD e condizioni delle ossa mascellari in termini di densità ossea e status parodontale. Sono stati reclutati 70 pazienti che avevano eseguito una densitometria Dxa nell'ultimo anno e sono stati visitati per ottenere le informazioni relative allo stato parodontale oltre ad una ortopantomografia standardizzata. Specifici indici morfometrici sono stati misurati sulla radiografia panoramica.

In ultimo, una correlazione statisticamente significativa è stata dimostrata tra il BMD e una combinazione di due indici panoramici: il qualitativo "Klemetti index" e il quantitativo "mandibular cortical widht". È stata calcolata l'accuratezza di questi indici e la combinazione dei due ha mostrato un alto valore predittivo nell'identificare pazienti sani. L'analisi statistica applicata agli indici parodontali ha mostrato una correlazione di profondità di sondaggio maggiore di 5 mm e una perdita di attacco clinico di 3-4 mm con la condizione di paziente sano. Questi risultati controversi possono essere collegati alle limitazioni dello studio come la numerosità del campione o il bias legato al centro di reclutamento.

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1 INTRODUCTION

1.1 OSTEOPOROSIS

1.1.1 DEFINITION

Osteoporosis is a "systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture"(1). It is considered as a "silent" disease, which is often diagnosed belatedly, since most of the times it is not associated with clinical manifestations until a fracture occurs (2). Osteoporotic fractures normally involve weight-bearing portions of the skeleton such as spine, proximal femur and wrist and develop as a consequence of falls or low-impact trauma or even spontaneously. (3).

1.1.2 DIAGNOSIS

The diagnosis of densitometric osteoporosis is related to the measurement of bone mineral density (BMD), which is commonly defined in relation to a T- or Z- score, both of which are units of standard deviation (SD). The T-score describes the number of SDs by which the BMD recorded in the single patient differs from the mean value expected in young white healthy females, whilst the Z-score defines the number of SDs by which the BMD recorded differs from the mean value expected for people of the same age and sex. The World Health Organization (WHO) defines osteoporosis as a T score 2.5 or more below the young female adult mean (4-6), while osteopenia corresponds to a T score between -1 and -2.5. Tab. 1

Classification	*T score*
Normal	≥ -1
Osteopenia	-1 < T > 2.5
Osteoporosis	≤ -2.5
Severe or established osteoporosis	≤ -2.5 + one or more fractures

Tab. 1 WHO Operational Definition of Osteoporosis based on BMD measurement

The National Health and Nutrition Examination Survey (NHANES) III database of femoral neck BMD measurements in white women aged 20-29 years (7) has been recommended by WHO, IOF, ISCD and NOF as the reference database for calculating T-scores and, therefore, the femoral neck has become the reference site for osteoporosis diagnosis (8). Some guidelines promoted the evaluation of BMD at both hip and lumbar spine and suggested to consider the lower of the two values to place a diagnosis of osteoporosis (9), although the use of multiple sites does not seem to improve the prediction of fractures (10, 11)

Several techniques have been adopted for BMD measurement (single and dual photon absorptiometry, quantitative computed tomography, quantitative ultrasound, etc), but the most validated one is dual energy X-ray absorptiometry (DXA), owing to the sensitivity of X-rays absorption to the bone calcium content and the trivial amount of radiations delivered (12). When it is impossible to measure/interpret BMD at the hip or lumbar spine, it is advisable to consider the 33% distal radius (one third of the radius) as reference site (13).

Although the hip and lumbar spine are by far the most widely used sites for the diagnosis of osteoporosis, other sites are sometimes evaluated to measure BMD, such as the calcaneus, the phalanx and the radius. Several studies have also tried to measure jawbone mineral density and to correlate it with the density at other skeletal sites (see chapter 1.3).

Screening programs for osteoporosis differ between Countries. The WHO reports that there is indirect evidence supporting screening programs in women aged 65 or older, but no direct evidence supports widespread screening programs (14) and overall this idea is not feasible in country like Italy due to the large number of potential patient and the limits of national healthcare system. The National Osteoporosis Foundation (NOF) and International Society for Clinical Densitometry (ISCD) (15) recommend bone densitometry for:

- women aged 65 and older and men aged 70 and older

- post-menopausal women and men older than 50 years based on the risk factor profile
- post-menopausal women and men older than 50 years who had an adult age fracture, with the aim to diagnose and determine the degree of osteoporosis.

A different approach is suggested by the UK National Institute for Health and Clinical Excellence (NICE) (https://www.nice.org.uk/guidance/cg146), which recommends the following:

- do not routinely measure BMD to assess fracture risk without prior assessing it with Fractured Risk Assessment (FRAX) tool or QFracture
- consider fracture risk assessment in all women aged 65 and older and men aged 75 and older
- consider fracture risk assessment in women aged under 65 years and men aged under 75 years in the presence of risk factors
- do not routinely assess the risk in people younger than 50 years, unless they have major risk factors
- following risk assessment with FRAX (without a BMD value) or QFracture, consider measuring BMD with DXA in people whose fracture risk is in the region of an intervention threshold for a proposed treatment, and recalculate absolute risk using FRAX with the BMD value.

In Italy, the approach considers the BMD evaluation for women and men aged 65 70 and older or in women and men aged under 65 in the presence of risk factors

1.1.3 CLASSIFICATION

Traditionally, two types of osteoporosis have been distinguished: *primary* and *secondary* osteoporosis:

- *Primary osteoporosis* is a condition that does not develop as a consequence of concomitant diseases and includes *post-menopausal osteoporosis* (type 1) and *senile osteoporosis* (type 2).

Secondary osteoporosis is caused by certain concomitant diseases or medications that affect bone metabolism and bone mineral density. Tab. 2

Hypogonadal states		
Androgen insensitivity	Anorexia nervosa	Athletic amenorrhea
Hyperprolactinemia	Panhypopituitarism	Premature menopause (<40 yrs)
Endocrine disorders		
Central obesity	Cushing's syndrome	Diabetes mellitus (type 1 & 2)
Hyperparathyroidism	Thyrotoxicosis	
Gastrointestinal disorders		
Celiac disease	Gastric bypass	Gastrointestinal surgery
Inflammatory bowel disease	Malabsorption	Pancreatic disease
Primary biliary cirrhosis		
Hematologic disorders		
Haemophilia	Leukaemia and lymphomas	Monoclonal gammopathies
Multiple myeloma	Sickle cell disease	Systemic mastocytosis
Thalassemia		
Rheumatologic and immune diseases		
Ankylosing spondylitis	Other rheumatic and autoimmune diseases	
Rheumatoid arthritis	Systemic lupus	
Neurological and musculoskeletal risk factors		
Epilepsy	Multiple sclerosis	Muscular dystrophy
Parkinson's disease	Spinal cord injury	Stroke
Miscellaneous conditions and diseases		
AIDS/HIV	Alcoholism	Amyloidosis
Chronic metabolic acidosis	Chronic obstructive lung disease	Congestive heart failure
Depression	End stage renal disease	Hypercalciuria
Idiopathic scoliosis	Post-transplant bone disease	Sarcoidosis
Weight loss		
Medications		
Aluminium (in antacids)	Anticoagulants (heparin)	Anticonvulsants
Aromatase inhibitors	Barbiturates	Cancer chemotherapeutic drugs
Depo-medroxyprogesterone (premenopausal contraception)	Glucocorticoids (≥5 mg/d prednisone or equivalent for ≥ 3 months)	GnRH (Gonodotropin releasing hormone) agonists
Lithium Cyclosporine A and tacrolimus	Methotrexate	Parental nutrition
Proton pumps inhibitors	Selective serotonin reuptake inhibitors	Tamoxifen (premenopausal use)
Thiazolidinediones (such as Actos® and Avandia®)	Thyroid hormones (in excess)	

Tab. 2 Conditions, diseases and medications that cause or contribute to osteoporosis and fractures (from (Clinician's guide to prevention and treatment of osteoporosis 2014).

However, this distinction was meaningful when little was known about osteoporosis and most of the cases were defined as "primary" simply because the underlying causes were unknown. Nowadays, this distinction is considered simplistic and no longer satisfactory

(16). A significative amount of the cases of osteoporosis, especially in males, can now be ascribed to different risk factors (such as oestrogen deficiency, corticosteroid therapy or hypogonadism) acting at different levels in causing bone loss (16). Moreover, in 1998 the type 1 and 2 of primary osteoporosis were reviewed and since then the "unitary model of osteoporosis in postmenopausal women and ageing men" has been applied (17). According to this model, oestrogen deficiency is the leading cause of bone loss in women but gives also a major contribution in the slow and continuous bone loss experienced also by elderly men (18).

1.1.4 EPIDEMIOLOGY

1.1.4.1 EUROPE

In 2010, it was estimated that osteoporosis had a prevalence of 27.6 million in Europe (22 million women and 5.6 million men), with 3.5 million new fragility fractures recorded and related to osteoporosis. These data are expected to significantly increase in the future, owing to population growth and ageing (19). The prevalence of osteoporosis is the highest in Whites (or Caucasians) and the lowest in US Blacks, while Mexican-Americans fall in between the two groups (16). Approximately one in two Caucasian women and one in five men are expected to experience an osteoporosis-related fracture within their lifetime (20).

The sites most typically affected by osteoporotic fractures are vertebral bodies, proximal femur, proximal homerus and distal radius (19). The hip (proximal femur) fracture is considered the most serious one, since it almost always requires hospitalization and it is associated with a high morbidity (60% of patients show difficulties in at least one essential daily life activity after one year (21) and high mortality (24% of patients die within the first year (22). Osteoporosis-related fractures have been related to more disability-adjusted life-years (DALYs) lost than any type of cancer, with the exception of lung cancer and account for 0.83% of the worldwide burden associated with non-communicable diseases (23). In 2010 estimated the economic cost related to osteoporotic fracture

treatment, long-term care and pharmaceutical prevention in Europe equivalent to €26 billions, €11 billions and €2 billions, respectively.

1.1.4.2 ITALY

In a 2013 country-specific report of the ministry of health on epidemiology of the osteoporosis, the Italian population at risk of osteoporosis was considered to include men and women ≥50 years. The number of men and women ≥50 years of age amounted to 10,791,000 and 12,997,000 respectively in Italy in 2010.

In the population at risk, the number of individuals with osteoporosis—as defined by the WHO diagnostic criteria—was estimated at 3,790,000. The number of incident fractures in 2010 was estimated at 465,000. Incident hip, clinical spine, forearm and "other" fractures were estimated at 91,000, 71,000, 72,000 and 232,000 respectively. 69 % of fractures occurred in women. In the population over 50 years of age, the number of individuals with hip and vertebral fractures that occurred before 2010 was estimated at 517,000 and 539,000 respectively. Moreover, the number of causally related deaths in 2010 was estimated at 5,476 and, overall, approximately 53 % of deaths occurred in women, confirming the relative more elevated risk in osteoporotic males. Regarding the economic incidence in Italy, the cost of osteoporosis in 2010 was considered to consist of three components:

- cost of fractures that occurred in 2010 ("first year costs")
- cost of fractures sustained prior to year 2010 but which still incurred costs in 2010 ("long-term disability cost")
- cost of pharmacological fracture prevention including administration and monitoring costs ("pharmacological fracture prevention costs").

The cost of osteoporosis in 2010 was estimated at € 7,032 million. First year costs, subsequent year costs and pharmacological fracture prevention costs amounted to € 4,269 million, € 2,402 million and € 361 million respectively.

According to this report, the population above 50 years of age is expected to increase from 23.8 million in 2010 to 29.2 million in 2025, corresponding to an increase of 23 %. The total number of fractures was estimated to rise from 465,000 in 2010 to 598,000 in 2025, corresponding to an increase of 28 %.

1.1.5 PATHOGENESIS

It is intuitive to understand that whenever a disease causes bone loss, this must be due to an imbalance in bone metabolism, between the anabolic process of bone formation and the catabolic process of bone resorption. Following an increase throughout the puberty, bone mass peaks close to the end of the third decade of life, after which a slow and progressive phase of bone loss begins. Studies utilising quantitative computed tomography (QCT) have demonstrated a different behaviour of cortical and trabecular bone density across time (Fig. 1). The cortical BMD tends to remain stable until mid-life and starts decreasing significantly with ageing (in association with sex steroid deficiency). On the contrary, trabecular BMD begins to slowly decrease already during adulthood and continues throughout life, thus suggesting an oestrogen-independent component (24, 25).

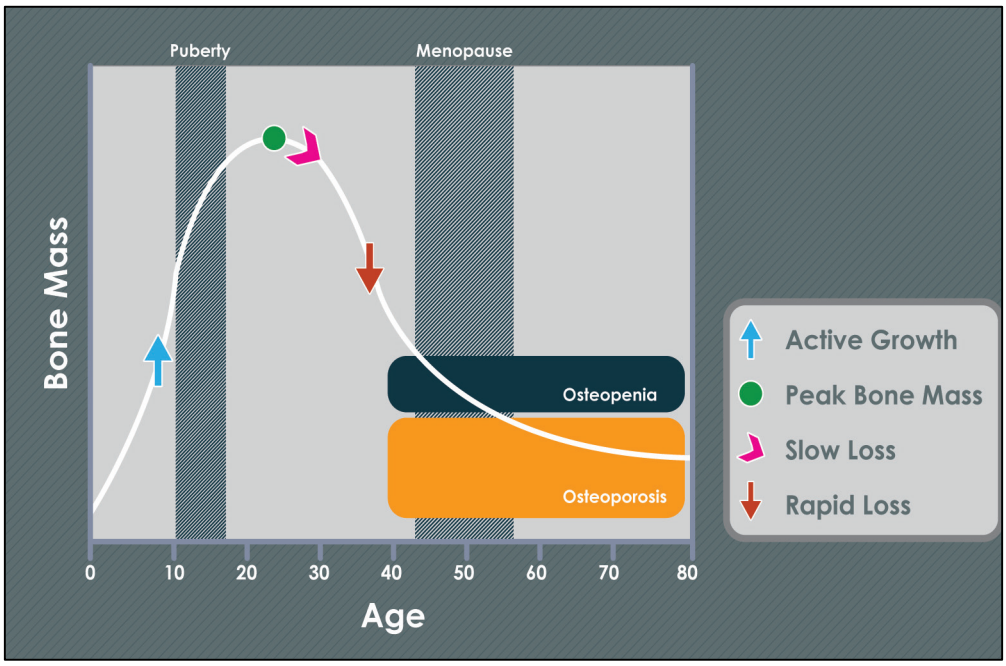

Fig. 1 Bone mass modification and behavior over time

The most critical event for women's bone mass is undoubtedly the cessation of ovarian function at menopause. Typically, at menopause there is an initial rapid phase of bone loss (2-4% of BMD rate decline a year) that lasts for the first 7-10 years. During this time, BMD may reduce in some women up to 25-33%, if not prevented by a specific therapy able to reduce bone loss, (ie, bisphosphonates, anti rank-L Ab, oestrogen or hormone replacement therapy). Afterwards, bone loss continues at a slower pace (approximately 1-2% BMD decline a year) for the remaining lifetime (17, 26-29). In the early phase, BMD loss involves mainly the cancellous bone and it seems to be triggered by *oestrogen withdrawal* and its subsequent effects on bone turnover and calcium levels. In elderly people, bone loss continues at a slower rate and affects both the trabecular and cortical bone (30)(Fig. 2). It is worth to remember that the cancellous bone is the first one to respond to any metabolic alteration, owing to its higher surface-to-volume ratio and denser vascularization (31).

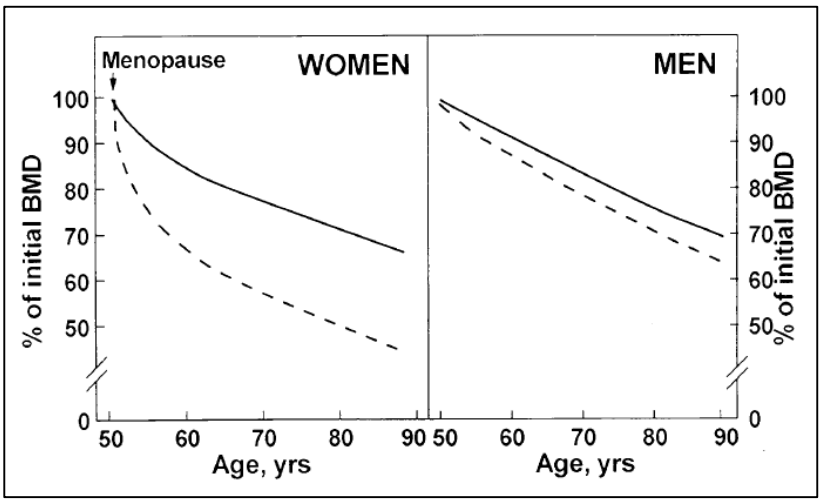

Fig. 2 Bone mass resorption rate in women (left) and men (right)

Oestrogen deficiency has a multiple effect on bone metabolism. Oestrogens act by binding to high affinity receptors (ERα and ERβ) and are able to directly modulate the activity of osteoblasts and osteoclasts (32, 33), but they can also indirectly influence bone physiology, owing to the presence of oestrogen receptors on other cells (such as stromal cells and cells of the immune system) (30). However, the rise in bone forming cells does not compensate the rise in bone resorption cells and this imbalance results in bone loss (34).

Another important function of oestrogen is to modulate the production of *pro-inflammatory cytokines*, such as interleukin 1 and 6 (IL-1, IL-6) and tumor necrosis factor (TNF)-alpha (35, 36). Oestrogen withdrawal is therefore associated with a pro-inflammatory status that stimulates bone resorption (37). There is some evidence, however, that this mechanism of increased bone loss could be limited only to the first early stage after menopause (38). It has been argued that oestrogen withdrawal can also increase the sensitivity of bone to parathyroid hormone (PTH), intensifying the bone resorption effect (39). This phenomenon of *secondary hyperparathyroidism* is common to both elderly men and women and seems one of the key mechanisms of the secondary slow phase of bone loss (17).

Another key element in the establishment of secondary hyperparathyroidism in aged people is *vitamin D deficiency*, since Vitamin D plays an pivotal role in maintaining the

appropriate calcium and phosphorus serum levels by stimulating intestinal calcium absorption (calcemic action), thus indirectly regulating bone mineralization. The biologically active form of Vitamin D, $1,25(OH)_2D_3$, was demonstrated to be a key modulator of osteoblast and osteoclast activity (40, 41).

Moreover, numerous studies have shown that ageing is associated with a significantly *reduced differentiation, activation and function of osteogenic cells* (42). Mesenchymal stem cells of bone marrow tend to differentiate more into adipocytes rather than osteoblast lineage, and this impaired osteoblast development/activation contributes to bone loss especially in the elderly (43, 44).

1.1.6 RISK FACTORS

Several modifiable and non-modifiable risk factors have been related to osteoporosis and are a relevant part of the pathophysiological mechanisms of these diseases. Among the modifiable ones the most important and more defined are: low body mass index and anorexia (BMI), alcohol consumption, poor nutrition (not only insufficient dietary calcium intake), smoking, vitamin D deficiency, and a sedentary lifestyle. Among the non-modifiable the most relevant are: risk factors, female gender, age, Caucasian ethnicity, early menopause and diseases affecting bone metabolism are the most largely studied (45, 46).

1.2 PERIODONTAL DISEASE

1.2.1 DEFINITION

1.2.1.1 ANATOMY OF THE PERIODONTAL TISSUES

The periodontium (peri = around, odontos = tooth) includes the following tissues: the gingiva, the periodontal ligament, the root cementum and the alveolar bone that is continuous with the alveolar process.

The primary function of the periodontium is to create the attachment between the tooth and the surrounding bone tissue and for this reason is considered “the supporting tissue of the teeth”. This apparatus creates a developmental, biologic and functional unit that can change due to age, functional modifications and oral environment alterations. Fig. 3

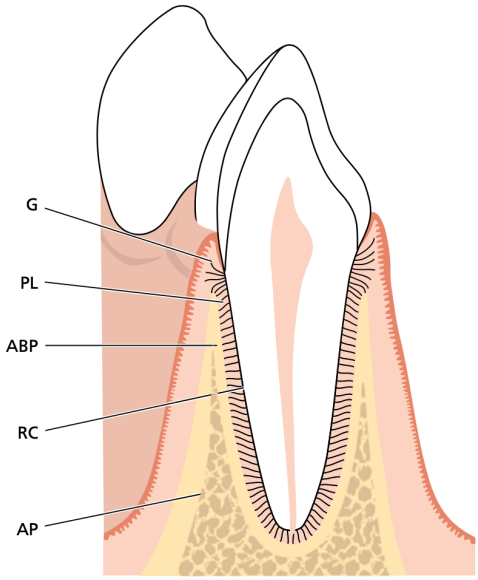

Fig. 3 The periodontium: gingiva (G), peridontal ligament (PL), root cementum (RC), alveolar bone proper (APB), alveolar process (AP)

1.2.1.1.1 Gingiva – macroscopic anatomy

The oral mucosa consists of the masticatory mucosa (gingiva and hard palate), the specialized mucosa (dorsum of the tongue) and the lining mucosa (the remaining parts). The gingiva covers the alveolar process of the bone and the cervical portion of the teeth. It can be subdivided in two different layers: the epithelial layer and the underlining connective tissue (lamina propria). Coronally the gingiva terminates with the free gingival margin, and in apical direction is continuous with the alveolar mucosa. The clearly recognizable lines that separates the two types of mucosa is called mucogingival junction. During an oral examination two types of gingiva can be differentiated: the free and the attached gingiva.

The free gingiva is bounded by the gingival margin coronally and the free gingival groove (corresponding to the cemento-enamel junction, CEJ) apically. A small invagination called sulcus is formed by the free gingiva all around the tooth and, when the periodontal probe is inserted in this sulcus towards the CEJ, a little pocket is created separating the tissue from the tooth surface. This socket is called “periodontal pocket” or “gingival pocket”. After complete/physiological tooth eruption and in condition of periodontal health this pocket is very thin and the soft tissues are in strictly contact to the enamel. In the interdental area, the shape of the gingiva is determined by the contact areas of the adjacent teeth, the CEJ, the tooth shape. This portion of gingiva is called “interdental

papilla” and can be pyramidal, i.e. in the anterior area where there are contact points between teeth, or more flattened in the posterior region where contact areas are more frequent.

The attached gingiva is bounded by the free gingival grove coronally and extend to the muco-gingival junction apically. After the MGJ the attached gingiva becomes continuous with the lining mucosa that covers all the oral cavity. The main feature that characterized the attached gingiva is the firm bonding with the underlining alveolar bone by connective tissue. For this reason, this type of gingival tissue is considered not movable, contrariwise, the alveolar mucosa is loosy bounded to the alveolar bone. Fig. 4

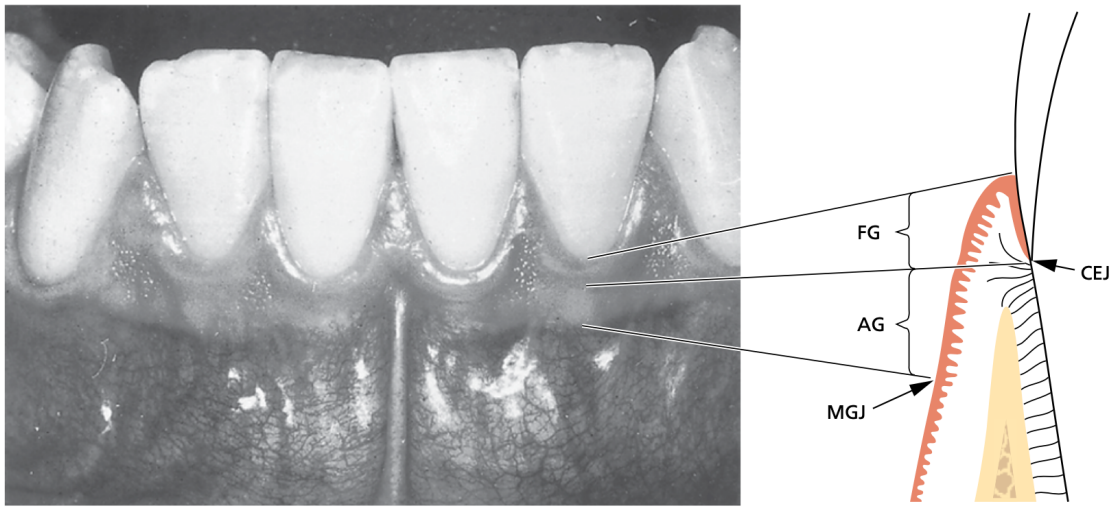

Fig. 4 Three parts of the gingiva: free gingiva (FG) that finishes at the cemento-enamel junction (CEJ), interdental gingiva, attached gingiva (AG). The demarcation line is the muco-gingival junction (MCJ)

1.2.1.1.2 Gingiva – microscopic anatomy

The gingiva comprises two types of structures, the epithelial tissue and the connective tissue. The epithelium covers all the gingiva and can be differentiated in different layers: oral epithelium (faces the oral cavity), oral sulcular epithelium (faces the tooth without contact) and the junctional epithelium (creates the contact gingiva-tooth). The contact between oral epithelium and the underlining connective tissue has a shape of digits. The areas of connective tissue that invade the epitelium are called connective tissue papillae, interspersed with epithelial ridges called rete pegs.

Oral Epithelium is keratinized, stratified, squamous epithelium that can be subdivided in basal layer (str. Basale), prickle cell layer (str. Spinosum), granular cell layer (str. Granulosum) and keratinized cell layer (str. Corneum). Oral epithelium is mainly made by keratine-producing cells (90%) and the remaining part is made by clear cells (malanocytes, langherans cells, merkel's cells, inflammatory cells).

The main tissue component of the gingiva is the connective tissue (lamina propria), made by connective tissue fibers, fibroblasts, vessels and nerves. All these structures are

embedded in an amorphous matrix that works as an environment for the cells. Different types of cells are present in the

connective tissue: fibroblasts that represent the 65 % of the entire population and are involved in the creation of the collagen tissue and the matrix; mast cells that are involved in the production of several components of the matrix and in the control of the blood circulation through the tissues; macrophages have a lot of syntetic functions and they are particularly numerous in the inflamed tissue. Neutrophilic granulocytes (polimorphonuclear leukocytes), lymphocytes and plasma cells are also present in connective tissue.

Connective tissue fibers, created by the fibroblasts, can be distinguished in 4 main categoiries: collagen fibers (the most essential components of the periodontium), reticulin fibers (present at the interfaces epithelium - connective tissue and endothelium - connective tissue), oxytalan fibers (scarse in gingiva but present in periodontal ligament), and elastic fibers (present in associacion with blood vessels in gingiva and without blood vessels in lining mucosa). Although, other collagen fibers are present, randomly or regularly distributed, and according to their direction and their insertion are distinguished in: circular fibers CF (present in the free gingiva with a circular shape all around the tooth), Dento-gingival fibers DGF (embedded in the cementum of the supra-alveolar portion of the root and connected to the free gingiva), dento-periosteal fibers DPF (embedded in the cementum but in connection with the attached gingiva. In the area between DGF and DPF can be present a line of depression called gingival groove), Trans-septal fibers TF (connecting supra alveolar cementum of adjacent teeth).

The connective tissue matrix is mainly produced by the fibroblasts, and is a medium in which the fibroblasts and other cells are embedded. Within the matrix there occur a non-stop exchange of nutrients, metabolites, water, electrolytes and other substances. The main components of the matrix are proteoglycans and glycoproteins.

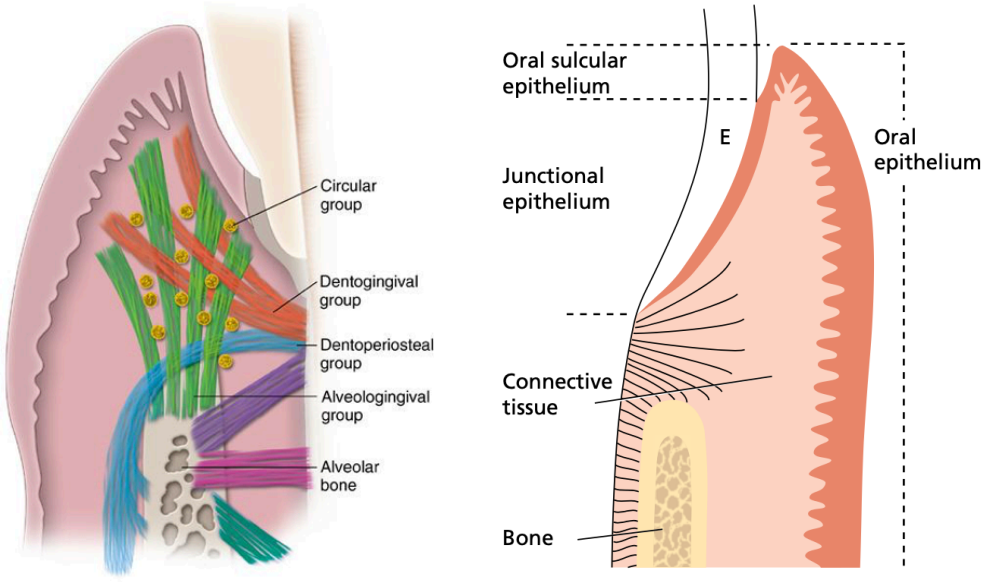

Fig. 5 The schematic drawing of the histologic section describing the composition of the gingiva and the contact area between the gingiva and the enamel.

1.2.1.1.3 Periodontal ligament

The periodontal ligament (PDL) is located in the slight space between root and alveolar bone and it is the structure responsible of the connection between the lamina dura and the tooth cementum. The ligament is a richly vascular, soft tissue that completely encircle the root and is in continuous with the lamina propria of the gingiva coronally. The width of periodontal ligament is about 0,25 mm (0.2-0.4 mm range) and his main function is to adsorb and distribute the masticatory load as well as the occlusal forces during other tooth contacts. According to the PDL characteristics (width, height, quality) the mobility pattern of teeth can be different.

Different bundles of collagen fibers constitute the periodontal ligament and It is possible to distinguish 4 different types depending on their orientation: alveolar fibers crest AFC, horizontal fibres HF, oblique fibres OF, apical fibers AF (**Fig. 6**). The principal collagen I fibers of the PDL are located between the cementum and the alveolar bone, and penetrate both the cementum and the bone surface. This type of fiber is called Sharpey's

fiber. In addition, the PDL contains oxytalane fibers that run around blood vessels with an apico-occlusal orientation and they are also inserted in the cementum.

The ligament is colonized by several cells as fibroblasts (along principal fibers), osteoblasts (on the bone surface), cementoblasts (on the cementum surface), osteoclasts, epithelial cells as well as nerve fibers. Epithelial cells are called Rest of Mallassez and are organized in clusters on root surface. These cells are a remnant of the Hertwig's epithelial root sheath. These cells are still vital but with a poor metabolism.

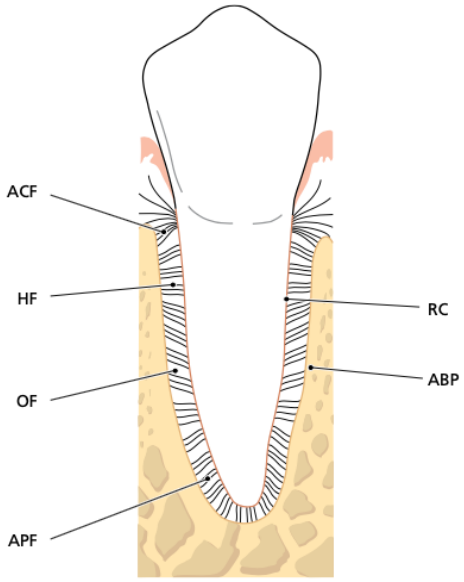

Fig. 6 Schematic draw of periodontal ligament with fibers orientation: alveolar bone proper (ABP), root cementum (RC), alveolar crest fibers (ACF), horizontal fibers (HF), oblique fibers (OF) and apical fibers (APF)

1.2.1.1.4 Root cementum

The cementum is a tissue covering the tooth root characterized by several bone-like features. It is a mineralized highly specialized structure without blood vessels and innervation. Cementum undergoes a continue reposition during life increasing in thickness by the addition of new layers but, as opposed to the bone, does not present alternated periods of resorption and apposition. It is formed by collagen fibred embedded in an organic matrix like other mineralized tissues and contains more or less

as much hydroxyapatite as the bone (65% vs 60%) in its mineralized composition. The two functions of the cementum are: linking the periodontal ligament to the root and contribute to the repairing processes of the root in case of damage. Root cementum can be divided in three different types: Acellular extrinsic fiber cementum AEFC (situated coronally and in the middle portion of the root and contains the Sharpay's fibers), Cellular mixed stratified cementum CMSC (in the apical third and in the furcations), Cellular intrinsic fiber cementum CIFC (found in resorption lacunae). Sharpey's fibers constitute the extrinsic fiber system of the cementum and are provide by fibroblasts, contrarywise, the intrinsic fiber system is made by bundles orientated mainly parallel to the long axis of the tooth and is produced by cementoblasts. Sharpey's fibers are constituted by a non-mineralized central part and two mineralized termination that are embedded into cementum and bone.

Cementum, throughout the life, becomes wider in the apical portion that can reach the width of 150-250 microns, in comparison to the coronal area where the thickness is about 20-50 microns.

1.2.1.1.5 Alveolar bone

The alveolar process is the portion of jawbones that include and support the teeth. It develops at the same time of the development and eruption of the teeth. The alveolar bone composes the attachment structure of tooth in combination with periodontal ligament and root cementum. The main function of the apparatus is to adsorb, distribute and modulate the masticatory and occlusal load. The walls of the socket that contains the teeth are covered by cortical bone, while the areas between walls and between sockets of adjacent teeth are constituted by cancellous bone, full of trabeculae (**Fig. 7**). In maxilla, bone walls of the sockets are thinner at the buccal aspect if compared to the palatal aspect and the thickness can vary in different areas of the mouth. In posterior area of the mandible the bone wall in the buccal aspect is thicker than the bone in lingual area, this is emphasized by the presence of the linea obliqua at level of second and third molars. In general, sockets walls become thinner from molar region to anterior area. The layer of

bone in the socket in which Sharpey's fibres are inserted is called bundle bone, a type of bone with some cementum-like features and a high turnover rate. The alveolar bone is constantly renewed as response to functional stimuli with a non-stop resorption-reposition pattern. These physiological functional demands include masticatory loading, occlusal forces or teeth movements. The cells involved in this process of resorption are osteoclasts, a giant cell type specialized in mineralization matrix brakeage by the release of acids. The continuous remodeling pattern affects cortical as well as cancellous bone, after bone resorption acted by osteoclast, the newly formed bone is deposed by osteoblasts and a new Bone Multicellular Unit BMU (the "structure" composed by all the cells and vascular supply necessary for bone remodeling) is created.

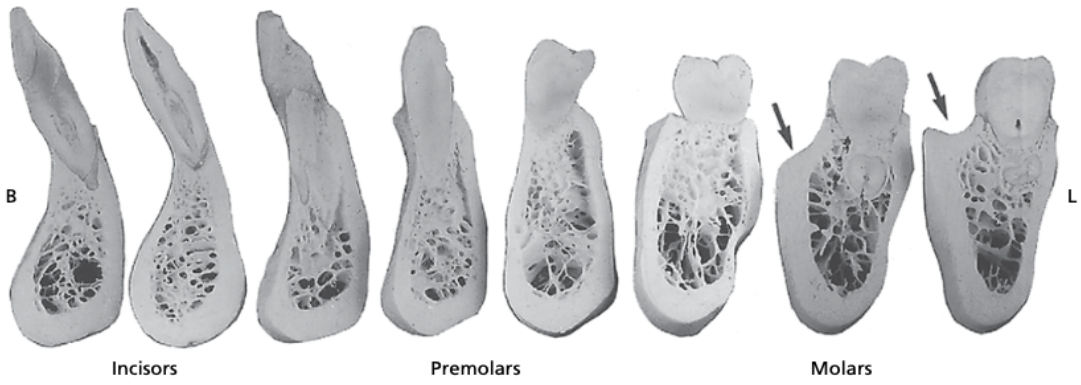

Fig. 7 Vertical sections through various regions of mandibular dentition. The bone wall at the buccal (B) and lingual (L) aspect

1.2.1.1.6 Blood supply of the periodontium

Hard and soft tissues of the maxilla and the mandible are supplied by a complex system of blood vessels rather than single groups of vessels. In fact, oral cavity is characterized by a packed network of anastomoses. Periodontal tissue and teeth is vascularized by vessels from the internal maxillary artery(IMA), that dismisses the internal alveolar artery (IAA) in the maxilla and in the mandible. In correspondence of each tooth the dental artery (DA) originates from the IAA and carries on towards the dental apex and, before

entering the dental socket, dismisses the intraseptal artery (AI) with rami perforantes (RRP) that compenetrates the periodontal ligament. Beneath the junctional epithelium there is a fine mesh network of small vessels without loops called dento-ginigival plexus (DGP).

Gingiva, contrarywise, receives the blood supply mainly through supraperiosteal vessels as terminal branches of a group of arteries: the sublingual artery, the mental artery, the buccal artery, the facial artery, the greater palatine artery, the infraorbitarial artery and the posterior superior dental artery. This network creates the subepithelial plexus (SP) in correspondence of free and attached gingiva. The plexus is characterized by several capillary loops projected from connective rete pegs to the epithelium. Free gingiva vascularization in completed and integrated by terminal branches coming from the periodontal ligament in anastomoses with supraperiosteal and bone vessels. Fig. 8

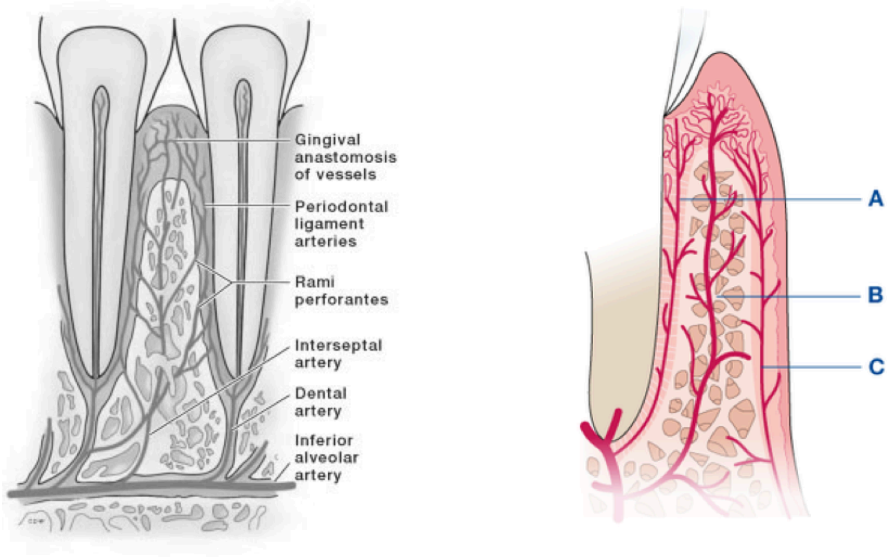

Fig. 8 The schematic draw of the blood supply to the teeth and the periodontal tissues: the intraseptal artery (B), periodontal ligament artery (A) and supraperiosteal vessels (C)

1.2.1.1.7 Lymphatic system of the periodontium

The lymphatic structures in periodontium are not easily identified because of the dimension. In fact, the lymph capillaries that form an extensive network in connective

tissue are characterized by a wall form by a single layer of endothelial cells. Before the entrance in the blood stream, the lymph passes through a group of lymph nodes in which is filtered and supplied with lymphocytes. All periodontal lymphatic drainage is collected in head and neck nodes. For the mandible, the drainage from the anterior gingiva and lips is collected in the submental nodes and for buccal/lingual gingiva of premolar/molar areas to the submandibular nodes. Regarding the maxilla, the palatal gingiva is drained to the deep cervical nodes and the buccal gingiva is drained to the sub mandibular nodes. Except for the third molars, that drain to the jagulogastric node, and for the mandibular incisors, that drains to the submental nodes, all the teeth and the relative periodontium are drained to the submandibular lymph nodes. Fig. 10

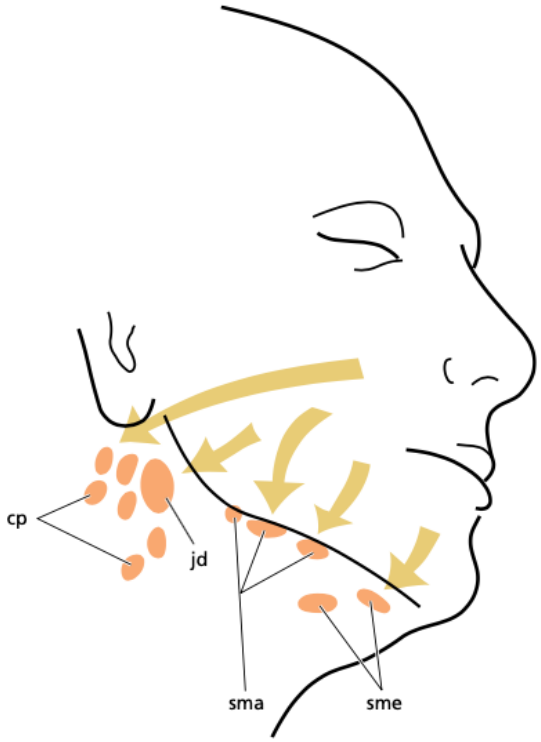

Fig. 9 Schematic draw of the lymphatic system: Jagulodigastric node (JD), deep cervical nodes (CP), sub-mandibular nodes (SMA) and submental nodes (SME)

1.2.1.1.8 Nerves of the periodontium

Periodontium contains nociceptors and mechanoreceptors that can transfer the information about pain, touch and pressure. These nerves have the trophic center in the semilunar ganglion and comes to the periodontium with trigeminal nerve and its three branches. The presence of a very thin metal foil can be identified by the periodontium (10-30 microns). Periodontal receptors together with the proprioceptors in muscles and tendons can be considered the masticatory system of the jaws playing a key role in regulation of chewing and occlusion movements.

The teeth of the upper jaw are innervated by the superior alveolar plexus, and in the lower jaw by the inferior alveolar nerve. For the periodontium the nervous system is the same of the dental system but the small nerves that go from the main nerve to the periodontal space follow the same course of the corresponding blood vessel.

In the maxilla, the gingiva of buccal aspect of incisors/canines/premolars is innervated by superior labial branches from the infraorbital branch, while the molar gingiva on the buccal aspect is supplied by the posterior superior dental nerve. The palatal gingiva is innervated by the greater palatal nerve except for the incisors area, served by the long sphenopalatine nerve. Regarding the mandible, the lingual gingiva is innervated by the sublingual nerve (terminal branch of the lingual nerve). In the labial aspect of the incisors and canines the gingiva is supplied by the mental nerve, while the molar area by the buccal nerve but, in the premolar region the two nerves frequently overlap. Fig. 10

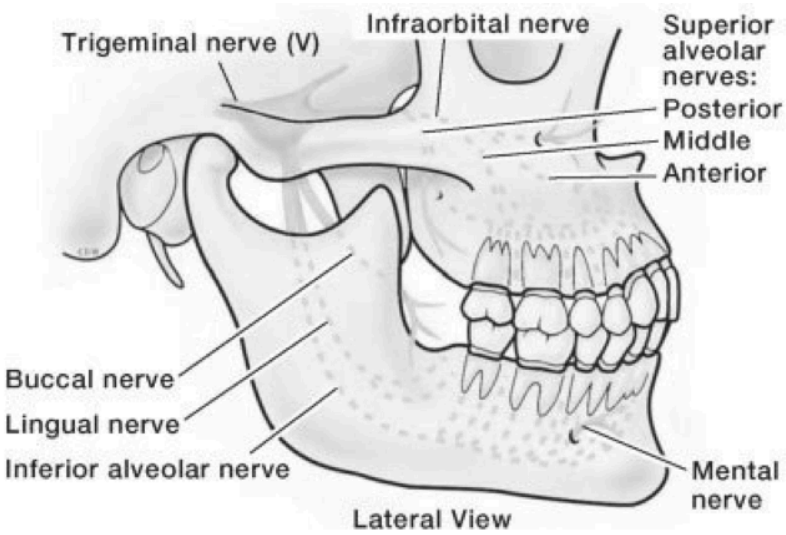

Fig. 10 Schematic draw of the innervation of the jaws

1.2.1.2 FEATURES OF PERIODONTAL DISEASE

Periodontal diseases are a class of infections characterized by the presence of microorganisms that colonize the tooth surface at or below the gingival margin. While billions of microorganisms colonize continually the tooth, most sites in most patients do not show loss of supporting tissues at any time. The relationship between periodontal microbiota and the host is in balance normally, but sometimes, a specific group of bacteria takes over the balance and lead to periodontal damage. Like other human infection, periodontal disease is characterized by the presence of periodontal pathogens, but is of primary importance to consider that this condition is necessary but not sufficient to a tissue destruction. In a large number of individuals in which the teeth are colonized by periodontal pathogens at both levels (supra or sub gingiva), the signs of periodontal suffering are absent and this is not an anomaly. In the same way of all the other infection in the human body, where different bacterial species have different tissues specificities and targets, periodontal disease appear to be caused by a restricted number of pathogens acting alone or in combination. These species include: Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Campylobacter rectus, Eubacterium

nodatum, Fusobacterium nucleatum, Peptostreptococcus micros, Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Streptococcus intermedius and Treponema(47).

The peculiar feature of periodontal infection is the presence of a “unusual anatomic feature that a mineralized structure, the tooth, passes through the integument, so that part of it is exposed to the external environment while part of is within the connective tissue”. The microorganisms that cause the disease reside in a biofilm that exist on tooth or on the epithelial surface. The major characteristic of the diseases is that they are caused by bacteria that reside in a biofilm outside the body and their treatment, as well as the diagnosis and the prognosis, is complex.

In general, periodontal diseases are a class of paraphysiologic conditions or pathologies characterized by bacterial colonization, soft tissue inflammation and/or soft and hard tissue destruction.

1.2.1.3 EXAMINATION METHODS

Examination of periodontal conditions consists of clinical assessment of inflammation in periodontal tissues, recording of probing depth and clinical attachment levels, besides a radiographic assessment of alveolar bone. Fig. 11

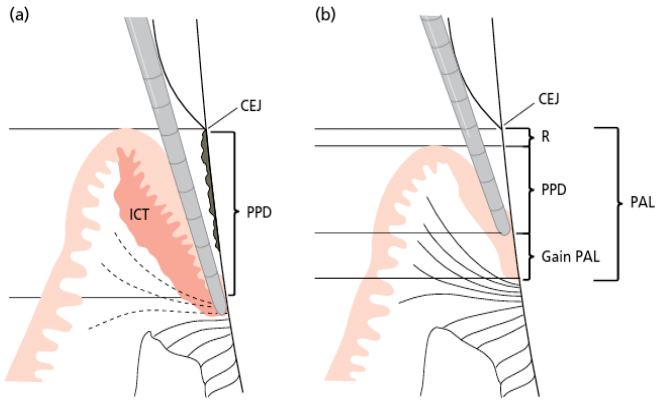

Fig. 11 Probing depth in a compromised (left) and healty (right) peridontal pocket

1.2.1.3.1 Inflammation assessment

Presence of inflammation at gingival margin in evaluated inserting a metal probe to the bottom of a gingival pocket according to the principles of the Gingival Index published by Loe in 1967 where the complete absence of inflammation at the margin was recorded as 0, the light tendency to inflammation with a change in color and appearance was recorded as 1, a slight bleeding after probing was recorded as 2 and the spontaneous bleeding was 3 (48). A similar score was set to record the presence of plaque where 0 was recorded in absence of deposits, 1 in presence of plaque visible moving the periodontal probe along the margin, 2 when the plaque was clearly visible and 3 if the plaque was abundant (49).

Later, simplified variants of these types of scoring have been proposed and used (50). These "new" scores are based on the dichotomy between presence or absence of bleeding and plaque. In this way, 0 records the absence and 1 records the presence of bleeding/plaque ("dichotomous scores"). The bleeding has to start within 15 seconds after the probing and this is called bleeding on probing (BOP). In case of a full mouth assessment six sites per tooth are probed and a Bleeding on Probing index can be recorded. The mean BoP score is given as a percentage, and this index can be used as a negative predictor of periodontal disease(51). Likewise, if the Plaque is assessed in a full mouth examination, a Plaque Index can be easily recorded. These two indexes can be easily used in order to analyse and compare the periodontal inflammation status at different observation periods. Fig. 12

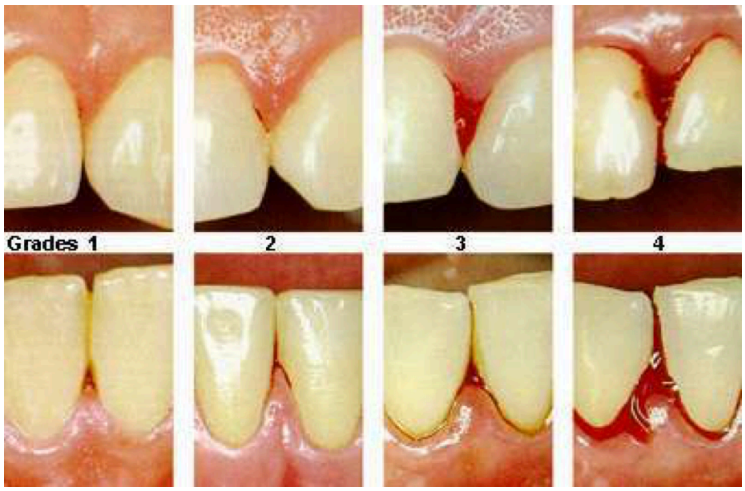

Fig. 12 Different degrees of inflammation in a periodontal tissue

1.2.1.3.2 Periodontal support assessment

A variety of examination index has been proposed over the years. In order to evaluate the amount of tissue lost and to identify the apical extension of the inflammatory lesion, the following parameters should be recorded:

- Probing pocket depth (PPD)
- Clinical attachment level (CAL)
- Furcation involvement (FI)
- Tooth mobility (TM)

Nowadays, loss of periodontal support is assessed by recording of periodontal probing depth (PPD), defined as the distance between the gingival margin to the location of the tip of a periodontal probe, with a standardized tip diameter of 0,4/0,5 mm, inserted in the pocket with moderate force. In a periodontal chart PPD<4mm are marked in black while deeper sounding with PPD>4mm are red. Red figures represent the diseased sites both from a severity and extension point of view. Several studies have demonstrated that PPD is the most valuable tool in clinical attachment loss prediction. In particular PPD associated to BOP+ has the higher level of predictability at 42 months (52), moreover,

residual pockets of 7mm have a predictability of 50% at 5 years and an increase of PPD>1mm has a predictability of 80% at 5 years (53).

Instead, clinical attachment level (CAL) is defined as the distance from the cemento-enamel junction (CEJ) and the tip of the probe. In a full mouth examination, a number of six points per tooth of the entire dentition are measured by probing (buccal, lingual, interproximal). In case of recession, the distance between free gingival margin and the CEJ turns negative and the height of the recession has to be added to the PPD to determine the CAL.

In the progression of periodontal disease on multi-rooted teeth, the destructive process can involve the structures around the furcation area. Furcation involvement is assessed from all the entrances of possible periodontal lesions of multi-rooted teeth with a curved periodontal probe graduated at 3 mm called “Nabers Furcation Probe”, and depending on the penetrating depth, FI are classified as (54):

- F1: horizontal PD < 3 mm from one or two entrances
- F2: horizontal PD > 3 mm in at the most one furcation entrance or in combination with a degree F1
- F3: horizontal PD > 3 mm in two or more furcation entrances (through-and-through)

Afterwards, in 1984 the classification has been integrated taking into account the vertical distruction of periodontal support and three classes can be defined besides the horizontal penetration deegres (55)(**Fig. 13**):

- Class A: 1-3 mm of PD
- Class B: 4-6 mm of PD
- Class C: > 7 mm of PD

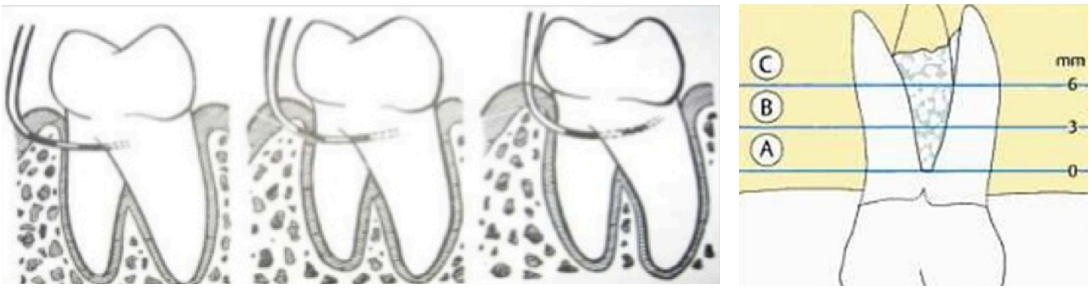

Fig. 13 Furcation sounding: The Nabers probe enters the furcation according to horizontal the bone destruction (F1, F2, F3, left). The grading of vertical bone resorption: A if F<3mm, B if 3mm<F<6mm,C if F>6mm (right)

The continuous loss of periodontal supporting tissue over time may result in increased tooth mobility, as well as the occlusal trauma. Increased tooth mobility has been classified in 1950 by Miller (56):

- Degree 0: Physiological mobility of 0,2-0,1 mm in a horizontal direction
- Degree 1: increased mobility at least 1 mm in a horizontal direction
- Degree 2: visually increased mobility exceeding 1 mm in a horizontal direction
- Degree 3: severe mobility of the crown in both horizontal and vertical direction (no function)

Is crucial to identify the cause of the mobility, in order to plan the proper therapeutic approach, in fact several factors could lead to an increase tooth mobility like overloading, periapical lesions, periodontal surgery, periodontal disease. Fig. 14

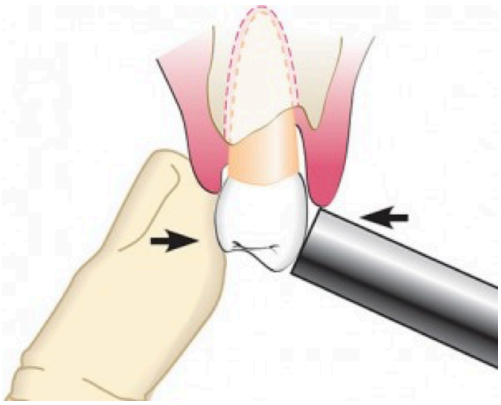

Fig. 14 Tooth mobility assessment

1.2.1.3.3 Radiographic evaluation

Radiology is widely used in periodontology but the limitations in supporting tissue loss evaluation are well known (57). Assessment of bone loss in radiographs can be used to evaluate: the presence of lamina dura, the width of periodontal ligament, the morphology of bone crest, the distance between CEJ and bone crest, the shape/distance of the roots. In general, radiological assessment is considered a useful tool mainly to perform epidemiological studies, rather than in clinical examination, where the periodontal probe remains the most reliable appliance. Fig. 15

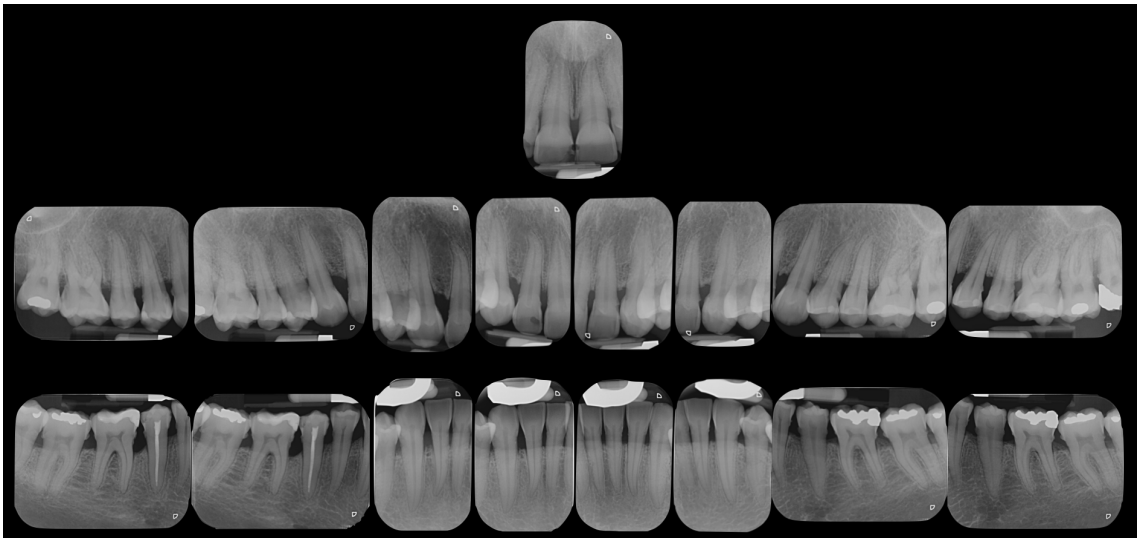

Fig. 15 Complete apico-periapical status

1.2.2 DIAGNOSIS OF PERIODONTAL DISEASE

Based on the information regarding the condition of the various periodontal structures (gingiva, periodontal ligament, alveolar bone) which has been obtained through the comprehensive examination listed above, a classification as well as a diagnosis for each tooth regarding the periodontal conditions may be given. Four different tooth-based diagnosis may be used:

- Gingivitis: teeth that show bleeding on probing but the sulcus depth remains at a physiological level of 1-3 mm irrespective of the level of clinical attachment. The diagnose of gingivitis characterized lesions confined to the gingival margin.
- Periodontitis “mild-moderate”: gingivitis in combination with attachment loss is termed “periodontitis”. If the PPD do not exceed 6 mm, a diagnosis of mild-moderate periodontitis is given irrespective of the morphology of periodontal lesion. This diagnosis can be applied to teeth with horizontal bone loss, representing suprabony lesions and/or vertical or angular bone loss with infrabony defects (one/two/three walls defects or craters).
- Periodontitis “advanced”: If PPD exceeds 6 mm, a diagnosis of advanced periodontitis is given irrespective of the morphology of periodontal lesion. The distinction with the mild-moderate is only based on the PPD, in fact horizontal as well as vertical defects can be present.
- Periodontitis interradicularis: This is the periodontitis characterized by the involvement of the furcation area. If a furcation is involved with a PPD<4 mm it is considered mild moderate, if PPD>4 is considered advanced periodontitis.

1.2.3 CLASSIFICATION OF PERIODONTAL DISEASE

The currently use d classification of periodontal disease was introduced by the 1999 International Workshop for a Classification of Periodontal Disease and Conditions and encompasses eight main categories, namely:

1. Gingival disease
2. Chronic periodontitis
3. Aggressive periodontitis
4. Periodontitis a manifestation of systemic disease
5. Necrotizing periodontal disease

6. Abscesses of the periodontium
7. Periodontitis associated with endodontic lesions
8. Developmental or acquired deformities and conditions

1.2.3.1 GINGIVAL DISEASE

It is demonstrated that inflammation of the gingiva induced by bacteria is the most common form of gingivitis (58), and the categorization of diseases affecting the gingiva requires evaluation of patient signs and symptoms, medical and dental histories, a clinical examination that includes the extend, distribution, duration and the physical description of lesions. The universal features of gingival disease include clinical signs of inflammation, signs and symptoms that are confined to the gingiva, reversibility of the disease by removal of etiology, the presence of bacteria-laden plaque to initiate and/or exacerbate the severity of the lesion, and a possible role as a precursor to attachment loss around teeth. The intensity of the clinical signs and symptoms of gingivitis will vary between individuals (59), as well as between sites within a dentition. The common clinical findings of plaque-induced gingivitis include erythema, edema, bleeding, sensitivity, tenderness and enlargement(58). Very specific features of gingivitis are the absence of supporting structure loss and the reversibility. According to Löe, it is important to underline that gingivitis becomes manifest only after days or weeks of plaque accumulation.

Gingival inflammation, clinically presenting as gingivitis, is not always due to accumulation of plaque on the tooth surface, this specific category of gingivitis is called “non-plaque induced inflammatory gingival lesions” (60). They may occur due to several causes, such as specific bacterial, viral or fungal infection without an associated plaque-related gingival inflammatory reaction. Gingival lesions of genetic origin are seen in hereditary gingival fibromatosis, and several mucocutaneous disorders (lichen planus, pemphigus, pemphigoids and erythema multiforme). Allergic and traumatic lesions are other examples of gingival disease non-plaque related.

1.2.3.2 CHRONIC PERIODONTITIS

Chronic periodontitis is considered to start as plaque-induced gingivitis, a reversible condition left untreated. The clinical features of CP include symptoms such as color/texture/volume alterations of the marginal gingiva, bleeding on probing from gingival pockets, increasing probing depth, loss of clinical attachment, recession of the gingival margin, loss of alveolar bone, root furcation exposure, increased tooth mobility, drifting and, eventually, exfoliation of teeth.

Chronic periodontitis has some peculiarities that can help the clinician in diagnose:

- CP is prevalent in adults but may occur also in children
- The destruction of the periodontal tissues see is commiserated with oral hygiene level, local predisposing factors, smoking, stress and systemic risk factors.
- Subgingival biofilm harbors a variety of bacterial species (biofilm composition varies between individuals and sites)
- CP is classified as localized when less than 30% of sites are affected, contrariwise is defined generalized
- Severity of chronic periodontitis at the site level can be classified based on the degree of clinical attachment loss as mild (1-2 mm), moderate (3-4 mm), severe (>5 mm)
- Although CP is initiated by plaque, host factors determine the pathogenesis and progression rate of the disease
- The rate of progression in mainly slow or moderate (but a faster progression can occur)

If gingivitis becomes manifest only after weeks of plaque accumulation, chronic periodontitis requires far longer periods of plaque and calculus exposure to develop (61, 62). Tissue destruction in CP does not affect all teeth evenly, but has a site predilection. As results in the same dentition, some teeth may be severely affected while other teeth can be almost free from the periodontal destruction. Regarding the

progression of the disease, a continuous slow process with periods of exacerbation (burst) seems the most concerted pattern. In general, it is important to know that factors associated to the disease can influence the disease progression. Furthermore, the extension and the severity of the disease are good predictors of future disease occurrence.

1.2.3.3 AGGRESSIVE PERIODONTITIS

Aggressive periodontitis (AgP) comprises a group of rare, often severe, rapidly progressive forms of periodontitis often characterized by an early age of clinical manifestation. In the absence of an etiological classification, aggressive forms of periodontal disease have been defined based on the following primary, universally present, features:

- non-contributory medical history
- rapid attachment loss and bone destruction
- familial aggregation of cases

And secondary, generally but not universally present, features:

- Amounts of microbial deposits inconsistent with the periodontal destruction
- Elevated presence of Aggregatibacter Actinomicetemcomitans and Porphiromonas Gingivalis
- Phagocyte abnormalities
- Hyper responsive macrophage phenotype, including elevated production of Prostaglandin E2 and interleukin-1B
- Progression of attachment loss and bone loss may be self-arresting
- <u>If localized aggressive periodontitis:</u>
    - Circumpubertal onset
    - Localized first molar / incisor with interproximal loss of attachment on at least 2 permanent teeth, one of which is a first molar, and involving non more than two teeth other first molar and incisors

    - Robust serum antibody response to infecting agent
- If generalized aggressive periodontitis:
    - Usually affecting persons under 30 years of age but sometimes can be older
    - Generalized interproximal loss affecting at least 3 teeth other than first molars and incisors
    - Pronounced episodic nature of the destruction of attachment an alveolar bone
    - Poor serum antibody response to infecting agent

Diagnosis of one of these forms requires the absence of systemic diseases that may impair host defenses and lead to premature exfoliation of teeth. The early manifestation of clinically detectable lesions is generally interpreted as being the expression of highly virulent causative agents or high levels of susceptibility of the individual patient, or a combination of two (63).

1.2.3.4 PERIODONTAL DISEASE AS A RISK FOR SYSTEMIC DISEASE

The evidence about the implication of periodontal disease as a risk factor for several systemic conditions such as cardiovascular disease, adverse pregnancy outcome, diabetes, and pulmonary disease, has emerged since 1990s (64).The findings gathered from the investigators worldwide in the last 60 years are very compelling, and it would certainly appear that periodontal disease is strongly associated with systemic conditions. For this reason, dentistry must focus on intervention studies to determine whether treating periodontitis will have a beneficial effect on systemic disease. Regarding cardiovascular disease, evidence demonstrating the beneficial effects of perio treatments on the disease outcome is limited and indirect (65). In considering adverse pregnancy outcomes, few studies provide evidence that preventive treatments aimed to reduced maternal periodontal infection may reduce the likelihood of preterm low birthweight infants (66).

Numerous epidemiologic surveys demonstrate an increased prevalence of periodontitis among patients with uncontrolled or poorly controlled diabetes mellitus. Moreover, diagnosing Diabetes type 2, investigators found a higher prevalence of clinical and radiological attachment loss for diabetics versus non-diabetic patients. Hence, it is clear that diabetes is a modifier or risk factor for periodontal disease (67). It is also demonstrated that periodontal infection reduction can improve glycemic control (68, 69).

There are a number of studies which examine the effect of treating oral infection in reducing the risk of pneumonia in high-risk populations (69). Recent reviews clearly indicate that when bacterial plaque is reduced in mouth of at-risk patients, the risk of pneumonia is reduced.

1.2.3.5 NECROTIZING PERIODONTAL DISEASE

Necrotizing gingivitis (NG) and necrotizing periodontitis (NP) are the most severe inflammatory disorders caused by plaque bacteria. These lesions, usually, run an acute course and are rapidly destructive and debilitating (70). Necrotizing disease is an inflammatory destructive periodontal condition characterized by ulcerated and necrotic papillae and gingival margin resulting in a punched-out appearance. The ulcers are covered by a yellowish with or grayish slough, termed “pseudomembrane”. Removal of the material results in a ulcerative lesion with massive bleeding. The lesions appear rapidly and are painfull, even if in the early stage pain could be moderate. The lesions are seldom associated with deep pockets because gingival necrosis is commonly associated to a crater-like interproximal bone destruction.

1.2.3.6 ABSCESSES OF THE PERIODONTIUM

Odontogenic abscesses include a broad group of acute infections that originate from the tooth and/or the periodontium. Such abscesses are associated with an array of symptoms including localized purulent inflammation in the periodontal tissues that causes pain and swelling. Depending on the origin of the infection lesions can be classified as periapical, periodontal and pericoronay abscesses. A classification has been proposed and included gingival abscesses, caused by impactation of foreign bodies, periodontal abscesses, either acute or chronic depending on a periodontal pocket, and pericoronal abscesses, for the incomplete eruption of a tooth. Periodontal abscesses classification is based on the etiology:

- periodontitis-related abscess: if an acute infection occurs from bacteria present in a deep periodontal pocket
- non-periodontitis-related abscess: when the infection originates from a different source, such as foreign body impaction or an altered root integrity.

1.2.3.7 PERIODONTITIS ASSOCIATED WITH ENDODONTIC LESIONS

Lesion of endodontic origin are significant as they frequently extend and manifest themselves in the attachment apparatus. Not only do these lesions produce signs and symptoms of inflammation in apical area but also along the lateral aspect of roots and in furcation areas. The fact that the periodontium and the dental pulp are anatomically interconnected also implies that exchange of noxious agents may occur in both directions (71). In general, periodontal-endodontic lesions are usually a tooth-related and not patient-related disease and, for this reason can occur either in periodontal or non-periodontal patients. Hence, this type of periodontal lesion is not considered a disease that can affect the patient but only the site.

1.2.4 EPIDEMIOLOGY OF PERIODONTAL DISEASE

Epidemiology is defined as “the study of the distribution of a disease or a physiological condition in human population and the factors that affect this distribution” (72). Based on this definition and previous epidemiological research in the medical field, in periodontal research, is it of primary importance the study of the *prevalence* of the disease in different populations (frequency, severity, changes).

1.2.4.1 PREVALENCE OF PERIODONTAL DISEASE

Since the last classification has been introduced in June 2018, a substantial part of existing literature on the prevalence and extend of periodontal disease in various populations is still based on an early classification of the disease. Currently, two principal forms of destructive periodontal disease are recognized – chronic periodontitis and aggressive periodontitis (73). It is relevant to attempt to appraise some key features relevant to the epidemiology of human periodontitis that underlie its core ‘identity’ as a bacterial biofilm-induced inflammatory disease. There is consensus that the epidemiologic hallmark of periodontitis is destruction of the tooth-supporting tissues manifested by clinical attachment loss and radiographic bone loss. A special feature of periodontitis is its site specificity, and an important consequence of this site specificity is that any measure of periodontitis as a pathologic condition which affects an individual rather than a tooth/tooth site must incorporate a measure of extent and also a measure of severity. To confuse the issue further, all biologic measurements, including attachment loss and bone loss, are subject to both temporal biologic variation and measurement errors (74). A universally accepted definition of the appropriate combination of extent and severity values to denote a ‘case of periodontitis’ has not been established and is one of the reasons why prevalence estimates of periodontitis vary considerably across studies. In developed countries, there is growing consensus that a valid examination of periodontal status should include full-mouth assessments of gingival/periodontal inflammation, probing depth and attachment loss, in other words measurements at six sites per tooth at all teeth present in the dentition and continuous, rather than

dichotomous, measures of extent and severity. According to the World Health Organization publication, 'Towards a Common Language for Functioning, Disability and Health: The International Classification of Functioning, Disability and Health', appears reasonable to assume that forms of high extent and severity are those most likely to be capable of negatively affecting the function and well-being of an individual. But if we try to understand why it has not been feasible to establish a universal definition of periodontitis for epidemiologic use, it is important to consider that the same severity level of periodontitis signifies vastly different prognosis at different ages: 6 mm of attachment loss at age 80 may be perfectly compatible with the retention of a functional dentition for life; however, the same magnitude of attachment loss affecting a teenager suggests a significantly worse prognosis with respect to tooth function and survival (75). Although periodontitis is often called a 'silent' disease because it is rarely associated with obvious signs and symptoms unless the disease has progressed to its terminal stages. A key issue in the present discussion is the current prevalence estimates of periodontitis. A systematic review including 72 studies and data from 291, 170 individuals ≥ 15 years of age from 37 countries estimated that the global prevalence of severe periodontitis in 2010 amounted to 10.8% (95% confidence interval: 10.1–11.6%), affecting 743 million people worldwide and representing the sixth most prevalent condition (76). The prevalence varied according to world regions, with southern Latin America and east Sub-Saharan regions scoring the highest prevalence, of 20%. We argue that the currently adopted epidemiologic methodologies/definitions that result in an almost ubiquitous prevalence of periodontitis indeed overestimate the occurrence of the disease that may actually put individuals at a true biologic, functional or psychosocial disadvantage. For this reason, nowadays, a multidimensional approach to the assessment of periodontitis would facilitate an improved understanding of its epidemiology and its consequences.

1.2.5 ETIOPATHOGENESIS OF PERIODONTAL DISEASE

1.2.5.1 MICROBIAL ETIOLOGY

Periodontitis is a complex disease with multiple component causes, some with their basis in genetics, some caused by epigenetic influences and others that are modifiable because they relate to patient behaviors, medications or environmental factors, all of which conspire to establish and propagate the periodontitis lesion (77).

1.2.5.1.1 Dental plaque and the biofilm concept

In general, all the interface surfaces of the body are exposed to colonization by a wide range of micro-organism and the establish microbiota live in harmony with the host. The accumulation and metabolism of bacteria on hard oral tissue is considered the primary cause of dental disease such as: caries, gingivitis, periodontitis, peri-implant infections and stomatitis. Plaque removal, in fact, leads to the disappearance of the clinical signs of inflammation (58). The term “infection” refers to the presence and multiplication of a micro-organism in body tissue. Dental plaque may accumulate supragingivally but also below the gingival margin, with a different composition of microbiota, that has been attributed to the local availability of blood products, pocket depth, redox potential and O2 (78). The ability to adhere to surfaces is a general property of almost all bacteria. The term “biofilm” describes the microbial community associated with a tooth surface , In the lower levels of most biofilms a dense layer of microbes is bound together in a polysaccharide matrix with organic and inorganic materials. Biofilm protect bacteria from antimicrobic agents, and, for this reason, treatments with antimicrobic agents without a mechanical plaque removal are unsuccessful. Literature data indicate that our host-associated polymicrobial communities, such as those found in the oral cavity, co-evolved with us and have become an integral part of who we are. Indeed, Roberts & Darveau argue that we should consider the microbiome as a human organ. (79)

1.2.5.1.2 Plaque-formation mechanism

Immediately upon immersion of a solid substratum into the fluid media of the oral cavity, or upon cleaning of a solid surface in the mouth, hydrophobic and macromolecules begin to adsorb to the surface to forma a conditioning film, termed “the acquired pellicle”. The film is composed of a variety of glycoproteins (mucins) and antibodies. Bacteria adhere variably to this coated surface. Behaviors of bacteria change once they become attached to the surfaces and the bacteria mass increases due to continued growth and adhering organisms, new adhesion and synthesis of polymers. Completely anaerobic conditions emerge in the deeper layer. Primary colonization is dominated by facultative anaerobic Gram-positive cocci and plaque collected within 24 hours mainly consists of streptococci (S. sanguis). In the next phase, Gram-positive filaments (Actynomices) are the predominant species. Veillonella, fusobacteria and other anaerobic gram-negative bacteria can attach due to the surface receptors despite of the poor ability to bind themselves to the first layer. Due to the difference of local environmental factors, structurally different types of plaque evolve at different locations. In summary, immediately following the immersion of hard, non-shedding surfaces into the fluid environment of the oral cavity, adsorption of macromolecules will lead to formation of a biofilm. Fig. 16

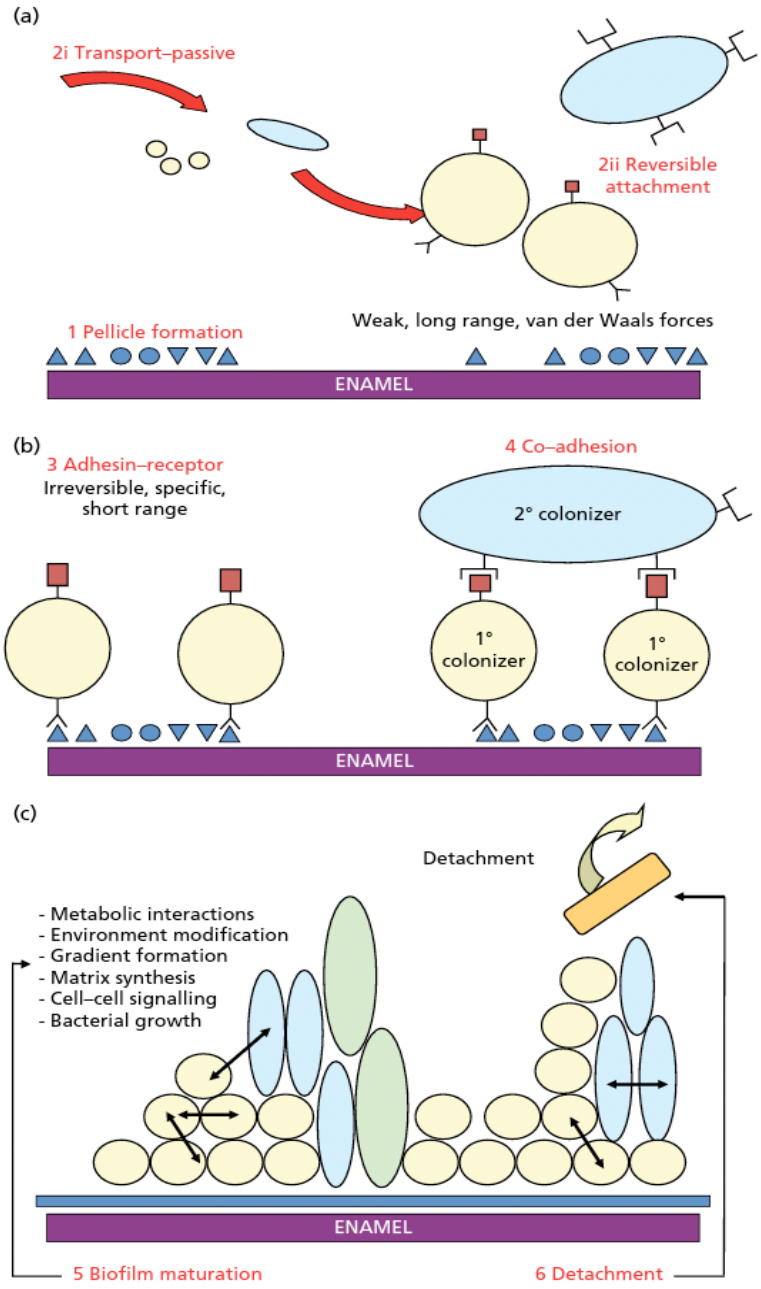

Fig. 16 Schematic representation of the different stages in the formation of dental biofilms. a) Pellicle forms on a clean tooth surface. Bacteria are transported passively to the surface. b) attachment becomes more permanent through specific stereochimical molecular interactions between bacterium and pellicle and secondary colonizers attach to the already attached primary colonizers. C) growth results in biofilm maturation.

1.2.5.1.3 Dental calculus

Dental calculus consists of mineralized bacterial plaque.

The degree of calculus formation is not only dependent on the amount of pacterial plaque present but also on the secretion of the salivary glands (80). Supragingivally, calculus can be recognized as a creamy-withish to dark yellow/brownish mass of moderate hardness. Subgingivally, instead, may be found by tactile exploration only, since its formation occurs apical to the gingival margin. Plaque mineralization varies greatly between and within individuals also within different regions of the oral cavity, and evidence of mineralization may already be present after few days (81). Supragingival plaque becomes mineralized saliva and subgingival plaque in the presence of inflammatory exudate in the pocket. It is evident that calculus represents a secondary product of infection and not a primary cause of periodontitis but it has to be realized that calculus is always covered by an unmineralized layer of viable bacterial plaque. It has been established that calculus roughness alone do not initiate gingivitis but can provide an ideal surface to further plaque accumulation and subsequent mineralization (82).

1.2.5.1.4 Periodontal pathogens

The criteria for defining pathogens of destructive periodontal disease initially were based on first and second Koch's postulate:

1. the agent must be isolated from every case of the disease
2. it must not be recovered from cases of other forms of disease or non-pathogenically

These criteria include association, elimination, host response, virulence factors, animal studies and risk assessment. However, microbiologists do not expect to find the pathogen in "all cases of the disease" because they cannot currency distinguish "all cases of a given disease". The criterion of elimination is based on the concept that elimination of a species should be accompanied by a parallel remission of disease. If a species is eliminated by treatment and the disease progresses, or if the level of a species remains high or

increases in a site and the disease stops, doubt would be cast on the species' role in pathogenesis.

The World Workshop in Periodontology (1996 Consensus Report) designated A. actinomycetemcomitans, P. gingivalis and T. forsythia as periodontal pathogens. The consensus summary, nowadays, is by no means exhaustive but does indicate that a growing literature suggests some reasonable candidates as etiologic agents of destructive periodontal disease.

The virulence of a microbial pathogen is generally defined as the degree of pathogenicity or ability of the organism to cause disease as measured by an experimental procedure. It represents a combination of highly complex parameters and depends upon both the relative infectivity of the organism and the severity of the disease produced. However, these two parameters of infectivity and disease severity are profoundly influenced by the nature and status of the host organism or the site of colonization in that host. For this reason, a breach in the normal defensive barriers of the host such as, trauma, immunosuppression, coinfection can increase the virulence of a given organism. The virulence determinant of a pathogen can simply be defined as those gene products which facilitate colonization, growth, and survival within the diseased host organism and spread to a new host. An emerging key property of these pathogens is the ability not only to overcome host defense but also to manipulate these systems to their own advantage. One example of this unique feature is the ability of both gram-negative and gram-positive bacteria A. actinomycetemcomitans and P. gingivalis to influence the pattern of cytokines expressions by host cells (83).

Aggregatibacter actinomycetemcomitans: one of the clearest associations between a suspected pathogen and destructive periodontal disease is provided by A. actinomycetemcomitans. Aa is a small, non-motile Gram-negative rod that forms small colonies (84). It was demonstrated that the majority of subjects with localized aggressive periodontitis had an enormously elevated serum antibody response to this species (85). Aa has been shown, in vitro, to have the ability to invade cultured human gingival epithelial cells, human vascular endothelial cells, and buccal endothelial cells in vivo (86). Aa has also been implicated in adult forms of destructive periodontal disease, but its role

is less clear. This species has been isolated in chronic periodontitis but less frequently and in lower numbers then from the lesion in Aggressive periodontal lesions subjects (87).

Porphiromonas gingivalis: is a second consensus periodontal pathogen that continues to be investigated. Pg is a non-motile Gram-negative anaerobic rod that forms black to brown colonies. Members of this species produce collagenase, gingival pain, an array of protease that destroy immunoglobulins, hemolysins, endotoxin, fatty acids, ammonia, hydrogen sulfide etc. Studies have strengthened the association of Pg with disease and demonstrated that the species is uncommon and in low numbers in health or gingivitis but more frequently detected in destructive form of disease. Pg has been also shown to induce elevated systemic and local immune responses in subjects with various forms of periodontitis (88). Like Aa, Pg has been demonstrated, in vitro, to have the ability to invade cultured human gingival epithelial cells, human vascular endothelial cells, and buccal endothelial cells in vivo (86).

Tannerella forsythia: Tf was described as the third periodontal pathogen in late 70's (89). The organism is a Gram-negative, anaerobic, spindle-shaped rod. Frequently is associated with F. nucleatum in subgingival sites (90). A feature of Tf is the presence of a serrated S-layer that has shown to mediate epithelial cells invasion and to induce cells apoptosis (86). This pathogen was thought to be uncommon but further studies with monoclonal antibodies have demonstrated that is more common than previously found and its levels were strongly related to increasing pocket depth. In addiction Tf was found more frequently and in higher numbers in actively progressing periodontal lesions than in active lesions (91).

1.2.5.1.5 The microbial complexes

The association of bacteria within mixed biofilm is not random, rather there are aspecific associations among bacterial species. The presence of specific microbial groups within dental plaque has been demonstrated (90).

Six closely associated groups of bacterial species were recognized (4 early colonizers and 2 predominantly Gram-negative periodontal pathogens complexes):

- Specific species of Actinomyces
- Yellow complex, consisting of members of the genus Streptococcus
- Green complex, consisting of Capnocytophaga species, A. actinomycetemcomitans serotype a, E. corrodens and Campylobacter concisus
- Purple complex, consisting of V. parvula and Actinomyces odontolyticus.
- Orange complex, consists of Campylobacter gracilis, C. rectus, C. showae, E. nodatum, F. nucleatum, F. periodonticum, Pe. Micros, Pr. Intermedia, Pr. nigriscens and S. costellatus.
- Red Complex, consisting of T. forsythia, P. gingivalis and Treponema denticola.

The last two complexes are comprised of the species thought to be the major etiologic agents of periodontal diseases. It is of primary importance to know that the microorganisms have an effect on their habitat, the periodontal tissues, and the habitat has a major effect on the composition, metabolic activities, and virulence properties of the colonizing microorganisms. Thus, modifications of the supra- and sub-gingival microbiota certainly affect the outcome, periodontal health or disease. Moreover, changes in the host or local habitat also affect the composition and the activity of the microbiota.

Perhaps the most influential factor on the composition of the subgingival microbiota is the periodontal disease status of the host (92). The major difference between health and disease, on average, was the increase counts, proportion and prevalence of the red complex species, T. forsythia, P. gingivalis and T. denticola in subjects with periodontal disease. Fig. 17

In addition, other putative periodontal pathogens of the orange complex were also more prevalent and in higher levels in periodontitis subjects. However, individuals with different form of the disease have different subgingival microbial profiles. Even subjects with the “same” periodontal disease in term of both clinical appearance and severity can exhibit quite different subgingival microbiotas.

In conclusion, if the biofilm is not disrupted frequently and is allowed to accumulate, the conditions within it start to favor bacterial species, such as Fusobacterium nucleatum,

that are capable of sensing and influencing their environment by employing chemical cues. Such 'quorum-sensing' organisms start to emerge and elicit a stronger host response, which, in turn, can lead to the development of gingival inflammation and increase the supply of certain nutrients that encourage the proliferation of traditional pathogens such as Porphyromonas gingivalis.

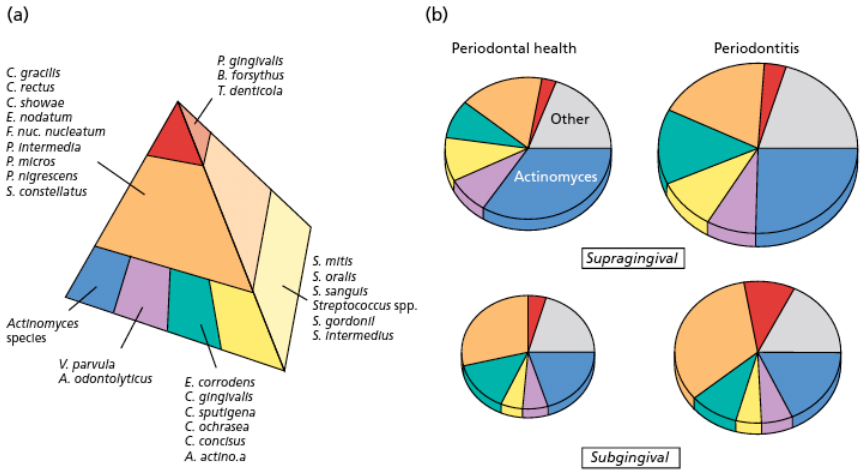

Fig. 17 a) Association among subgingival species. The different colors in the pyramid represent different bacterial complexes which are frequently detected in association with one another. The base of the pyramid represents the early stage of plaque development, whereas the apex contains those organisms thought to be the last species to become established in the microbiota. b) Pie charts of the mean percentage DNA count of microbial groups in supragingival plaque.

1.2.5.2 PATHOGENESIS OF PERIODONTAL DISEASE

The classical model of periodontal disease pathogenesis, developed by Page e Kornman in 1997 (**Fig. 18**) provides a key framework to underpin studies aimed at unraveling the complex interdependent relationships that exist both within the plaque biofilm and between the biofilm and the host response. We now recognize that a pathogenic biofilm is a necessary prerequisite for periodontitis to develop but in itself is insufficient to cause

the disease (93). Disease results from complex interactions between the biofilm and the inflammatory immune response, and it is the latter that is estimated to account for almost 80% of the risk of periodontal tissue damage (94).

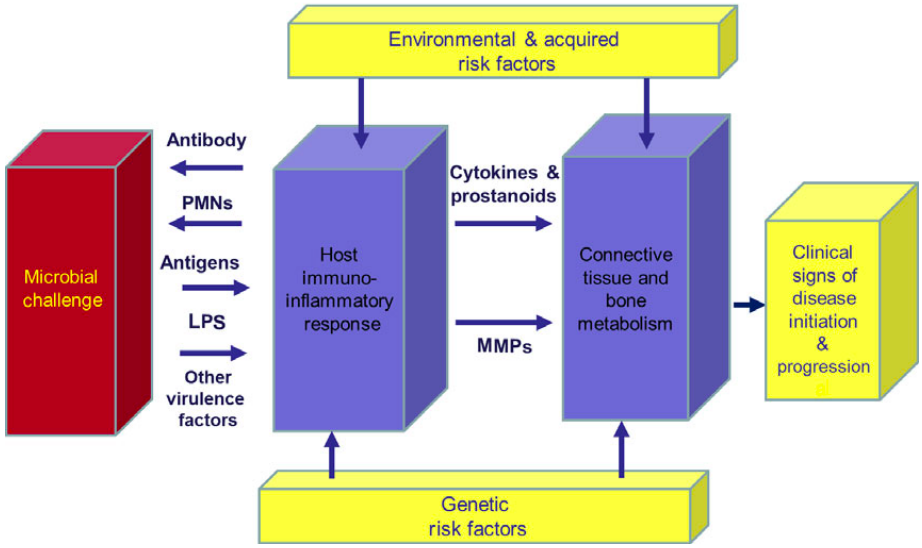

Fig. 18 Classical model of Page & Kornman, showing host–microbeinteractions in the pathogenesis of periodontitis. LPS, lipopolysaccharide; MMPs, matrix metalloproteinases; PMNs, polymorphonuclear neutrophils.

The periodontitis phenotype is characterized by an exaggerated, yet poorly effective and non-resolving, inflammation of the connective tissues supporting the teeth that leads to tissue destruction, rather than a specifically targeted, effective and self-resolving inflammatory immune response. One of the key changes in researcher perceptions of the infectious immune condition, which it is call periodontitis, is the realization that retaining or attaining clinical health requires a health-promoting biofilm within which symbiotic relationships exist between microorganisms and with the host response.

Moreover, in susceptible patients, incipient dysbiosis can trigger an inappropriate, and frequently excessive, host response, in which excess cytokines, reactive oxygen species (oxidative stress) and matrix metalloproteinases are generated and overwhelm their respective antagonists, resulting in collateral periodontal tissue damage. the chronic inflammatory state is characterized by attempts at healing (angiogenesis and fibrosis)

arising at the same time as inflammation, creating a rich nutritional environment for sustaining the dysbiosis and thus the pathogenic biofilm.

The colonizing microbes on mucosal surfaces, together with dangerous endogenous signaling molecules, such as extracellular ATP or extracellular DNA, activate the inflammasome, resulting in the subsequent secretion of the proinflammatory cytokines interleukin-1beta and interleukin-18. Inflammasome activation is mediated by caspase-1 activity, which also has an important role in the activation of cellular apoptosis. Reactive oxygen species are also required, and indeed periodontitis is characterized by oxidative stress, providing further opportunities for inflammasome activation, which may result in positive feedback, impacting upon the host response as well as on the chronicity of the inflammation (95). The release and secretion of proinflammatory cytokines activates polymorphonuclear leukocytes (neutrophils), which express various cell-surface receptors that bind to chemotactic stimuli and initiate downstream signaling sequences that lead to complex reactions and events, including cytosolic actin reorganization, shape changes and development of cellular polarity. The release of histamine and the activation of complement components C3a and C5a leads to vasodilatation, increased vascular permeability and slowing of blood flow within the respective capillary beds. The neutrophils then fall out of mildstream blood flow and contact the vascular endothelium, where they marginate and roll on the endothelial surface of the capillaries before binding firmly, via intergrin receptors, and moving out of the blood vessels into the tissues via diapedesis. Neutrophils belong to the fastest-moving mammalian cells, and once in the tissues they migrate along a chemotactic gradient that enables them to locate the site of infection and respond via receptor mediated phagocytosis and subsequent intracellular killing of the ingested bacteria (96).

A major role in this process is played by the lectin site of the leukocyte b2-integrin receptor (also known as CR3, Mac-1 and CD11b/CD18). Reports suggest that another potentially important receptor (delineated from other myeloid cells), lipopolysaccharide receptor (CD14), is only found in an inactive form. Eventually stored within the neutrophil, CD14 becomes expressed on the outer cell-membrane surface under extreme conditions. Together with neutrophil diapedesis and chemotactic migration toward the

site of bacterial infection, local capillaries also release an enhanced amount of serum as a result of the effects of histamine and complement C3a and C5a upon vascular permeability. The increased tissue fluid causes the tissues to swell and also increases the exudation of gingival crevicular fluid. Some pathogens are able to extend and prolong these inflammatory reactions in order to guarantee a continuous supply of host-derived nutrients.

Neutrophil activation leads to phagocytosis and intracellular killing of microorganisms, as well as to the release of enzymes, which may contribute to local tissue destruction. Enhanced release of elastase and other proteinases, such as collagenase, results in the depolymerization of tissue collagen fibers, thus increasing local tissue permeability. Fig. 19

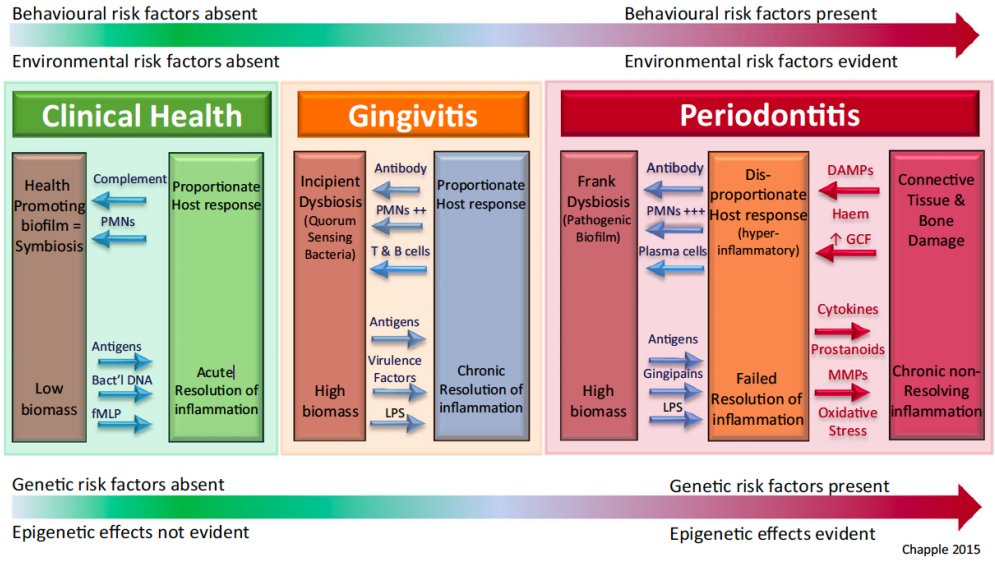

Fig. 19 Contemporary model of host–microbe interactions in the pathogenesis of periodontitis, in which the host response drives an incipient dysbiosis (gingivitis). If the biofilm is not disrupted/removed, frank dysbiosis results and perpetuates a chronic nonresolving and destructive inflammation. DAMPs, damage-associated molecular patterns; fMLP, N-formylmethionyl-leucyl-phenylalanine; GCF, gingival crevicular fluid; LPS, lipopolysaccharide; MMPs, matrix metalloproteinases; PMNs, polymorphonuclear neutrophils.

1.2.6 RISK FACTORS FOR PERIODONTITIS

There is an abundance of both empirical evidence and substantial theoretical justification for accepting the widespread belief than many diseases have more than a cause, in other words they have a multifactorial etiology (97). Consequently, in any particular instance when a causal relationship is investigated, the specificity of the relation between exposure to an etiological agent and effect (the necessity or the sufficiency of the condition) may be challenged. In the case of periodontal diseases, it is known that the presence of the microbial agent (which is defined as a necessary condition) is not always accompanied by signs or symptoms characteristic of that disorder. The *causal inference,* the process of drawing conclusions related to the cause of a disease, is a particularly complex issue in epidemiologic research. Hill in 1971 formalized the criteria that have to be fulfilled in order to accept a causal relation (98):

- Strength of association: the stronger the association between putative risk factor and the disease, the more likely it is that the anticipated causal relation is valid.
- Dose-response effect: the observation of the frequency of the disease increase with the dose of exposure to a factor
- Temporal consistency: to establish if the exposure to the causative factor occurred prior to the disease
- Consistency of findings: if several studies about the same relationship show the same results.
- Biologic plausibility: if the relationship should make sense in the contest of current biologic knowledge.
- Specificity of the association: if the investigated factor is found to be associated with only one disease and vice versa.

In the contest of periodontitis, numerous cross-sectional studies identifying potential risk factors are available, but a relatively limited number of longitudinal studies involve a multivariate approach to the identification of exposures of interest while simultaneously controlling for the effect of possible confounders. A review by Borrell and Papapanou,

has made a distinction between putative factors that are not amenable to intervention (non-modifiable background factors) and modiafiable factors (environmental, acquired, and behavioral) (99).

1.2.6.1 NON-MODIFIABLE RISK FACTORS

1.2.6.1.1 Age

The relationship between periodontitis and age is complex, even if it is clear that the prevalence of periodontal disease increases with age (100). Some authors challenge the relationship considering the "age effect" as the cumulative effect of the prolonged exposure to true risk factors (101). The association between periodontitis and age appears to be different for pocket depth and amount of clinical attachment loss. While there is a pronounced effect of increasing attachment loss with age, the effect on pocket depth is minimal (102). Since periodontitis is a microbially-induced inflammatory disorder, an age-dependent alteration in innate immunity likely contribute to more pronounced periodontal pathology in elderly individuals. In conclusion, an age-related, rather than an age-dependent, increased susceptibility to periodontitis in older people is biologically plausible.

1.2.6.1.2 Gene Polymorphisms

Evidence of genetic predispositions of certain individuals has been documented in classical twin and family studies. An association between a composite genotype based on specific polymorphisms in the interleukin1 (IL-1) gene cluster and severe periodontitis in non-smokers has been reported (). Similar work has investigated polymorphism in additional inflammatory genes, including the tumor necrosis factor (TNF) gene, the IL-6 gene, The IL-4 and the IL-10 gene. (103, 104)

The majority of cross-sectional studies report positive associations between the investigated polymorphisms and the extend and the severity of periodontitis. However, these results are not consistent across populations and between ethnic groups (105). The results of few longitudinal studies are also conflicting. Nevertheless, Lang has concluded

that IL-1 genotype-positive patients have a genetically determined hyper-inflammatory response that is expressed clinically in the periodontal tissues as increased prevalence and incidence of BOP during maintenance (51).
In conclusion, there is insufficient epidemiologic evidence to establish a polymorphism as true risk factor for periodontitis (106)

1.2.6.2 MODIFIABLE ENVIRONMENTAL, AQUIRED AND BEHAVIORAL FACTORS

1.2.6.2.1 Specific microbiota

The association between high levels of colonization by specific periodontal pathogens and the progression of periodontal disease has been corroborated by longitudinal data in untreated population (see *Etiopathogenesis of periodontal disease* chapter). Collectively, Data generated in the past decades have enhanced our knowledge of the role of specific periodontal bacteria as risk factor for periodontitis and have clarified that the intensity of the exposure to the specific microbiota rather than the presence of the pathogen is an important determinant of the clinical phenotype. Moreover, the virulence of the pathogen, and its ability to cause periodontal tissue damage and confer risk for disease progression may be entirely different among various clonal types within a single species. On the other hand, pathogen elimination from the subgingival microbiota results in improvements in clinical periodontal status. As widely demonstrated, an antimicrobial approach, including removal of subgingival plaque with or without the adjunct of antibiotics or antiseptics, followed by an adequate maintenance protocol, is the single most successful and consistent strategy in the treatment of periodontitis (107).

1.2.6.2.2 Smoking

The association between tobacco smoking and periodontitis was founded on the broad effects of multiple tobacco-related substances on cellular structure and function. Smoking has been shown to affect the vasculature, the humoral and cellular immune

responses, cell signaling processes, and tissue homeostasis (108). It is important to underline that the inferior periodontal status of smokers cannot be attributed to poorer plaque control or more severe gingivitis. In fact, smoking contributes to the formation of a dysbiotic biofilm, different from the non-smoker biofilm, characterized by a higher level of colonization by periodontal pathogens. Studies examining the effects of smoking on the outcome of periodontal treatment have demonstrates that treatment responses are modified by cigarette consumption, with current smokers exhibiting poorer responses than former or never smokers (109). These studies have confirmed the negative effect of smoking on the outcome of several periodontal treatments modalities, including non-surgical, surgical, and regenerative periodontal therapy (110). In contrast, smoking cessation was shown to have beneficial effect on periodontal status.

In conclusion, cigarette smoking appears to fulfill the majority of the required steps of the risk assessment process stipulated by Beck and is considered one of the major risk factors for periodontitis.

1.2.6.2.3 Diabetes Mellitus

The role of diabetes mellitus (DM) as a risk factor for periodontitis has been debated for decades (111). The adverse effect of DM on periodontal status appear to be particularly pronounced in subject with a long duration of DM and poor metabolic control (112). Studies have provided evidence of a dose-response relationship between poor metabolic control and the severity of periodontitis. The outcome of periodontal treatment in well-controlled diabetic patients is similar to that of non-diabetic subjects, while patient with poorly controlled DM display an inferior treatment outcome (113).

Several studies suggest a two-way relationship between DM and periodontitis. Beyond the observed increased severity of periodontal tissue destruction in subjects with DM, studies indicate a higher incidence of DM complications and poorer metabolic control of diabetes in periodontitis patients.

1.2.6.2.4 Obesity

The plausibility of a potential link between obesity and periodontitis has been suggested to involve a hyper-inflammatory state and an aberrant lipid metabolism prevalent in obesity, as well as the pathway of insulin resistance all of which may collectively result in an accelerated breakdown of the periodontal tissues (114)

A number of studies have indicated a positive association between obesity, defined as body mass index (BMI) > 30 kg/m2, and periodontitis. It is important to know that the majority of the publication is cross-sectional, and thus do not facilitate interferences on temporality or mechanisms, and the available eoidemiologic data are limited and not universally consistent, additional research on the role of obesity in periodontitis is warranted.

1.2.7 PERIODONTITIS AND OSTEOPOROSIS

Likewise osteoporosis, periodontitis is a silent disease, which often remains undiagnosed until late, when teeth become loose or an abscess develops. Interestingly, both diseases also share some risk factors, such as old age, smoking, and diseases/medications that may interfere with osseous healing (115). As a consequence, several studies have tried to investigate if these two diseases are linked and if osteoporosis can be considered a predisposing factor for the destruction of periodontal support.

Early cross-sectional studies of limited sample size and largely confined to post-menopausal women, have suggested that women with low bone mineral density are more likely to have gingival recession and/or pronounced gingival inflammation and clinical attachment loss (116).

A radiographic study by Pearson in 2002 reported a positive association between osteoporosis and periodontitis with a significant OR of 1,8 (95% CI 1,2 – 2,5). However, studies that have failed to demonstrate the correlation have also been published (117).

Based on these observation, it has been hypothesized that the systemic loss of bone density in osteoporotic patients may, in combination with other factors, provide a host system that is increasingly susceptible to inflammation-associated destruction of the periodontal tissues (118).Overall the data from longitudinal studies are conflicting, in fact some studies report a positive association between osteoporosis and clinical attachment loss in women with subgingival calculus but not if the calculus were supra-gingival (119). In these available studies, osteoporosis was diagnosed by applying different techniques, going from the old single and dual photon absorptiometry, to single or dual X-ray energy absorptiometry tha is the actual WHO gold standard, to ultrasound or quantitative computerized tomography. On the other side, periodontitis was assessed either radiographically, by measuring alveolar bone resorption, or clinically, by measuring clinical attachment loss or tooth loss. It is therefore intuitive to understand how heterogeneous the publications are and how difficult it is to draw meaningful conclusions. More recent systematic reviews of the available studies on osteoporosis and periodontitis concluded that the relationship between two conditions could be plausible but is still unclear (118).

1.3 OSTEOPOROSIS AND JAWBONES

1.3.1 SYSTEMIC AND JAW BONE LOSS

It is plausible to hypothesize that osteoporotic-induced systemic bone loss may also include bone loss at the jaws. Pre-clinical studies in ovariectomized animals reported that oestrogen deficiency could determine a decrease in bone volume and alterations in the trabecular structure of the mandibular condyle (120, 121), in the inter-radicular septa of molar alveolar bone (122, 123), an increase in mandibular cortical porosity (124) and a reduction in mineralization density, osteocyte lacunae, and osteocyte nuclear number (125).

Some clinical studies suggested that there is an increased alveolar bone resorption in osteoporotic versus non-osteoporotic edentulous patients (126, 127), and that medications affecting systemic bone density (like hormone replacement therapy and bisphosphonates) are associated with a slower loss of alveolar bone (128). However, other clinical evidences did not confirm the influence of systemic bone mineral density on the resorption of edentulous jaws (129-131). Several clinical studies investigated the relationship between bone density measured in different systemic skeletal sites and in the jawbones in subjects with different T scores. Although many of these studies found a positive correlation (132-139), others reported that jawbone density is only partially correlated to the density in other anatomic sites (140-142). There are also some limited evidences that did not found any differences in jawbone density between normal and osteopenic/osteoporotic subjects (143, 144). The heterogeneity between the available studies may have contributed to these conflicting results. In fact, different techniques to measure bone density were adopted, dentate and edentulous areas were often pulled together and populations with different demographic characteristics were evaluated without accounting for these important confounding variables.

Despite the contrasting results, during the past two decades, several studies have evaluated the accuracy of different quantitative and qualitative indices calculated on

dental radiographs (OPG) in identifying patients with reduced skeletal BMD (145-150). Amongst the linear indices, the following are the most popular ones:

1) Mandibular cortical width (MCW) or mental index (MI) or mandibular cortical thickness (MCT): It is usually measured in the mental foramen region, along a line passing through the middle of the foramen and perpendicular to the tangent to the lower border of the mandible (151).
2) Panoramic mandibular index (PMI): It is the ratio between the MCW and the distance from the lower border to the inferior edge of the mental foramen (152)
3) Antegonial index (AI): It is the mandibular thickness measured at the anterior border of the ramus (153)
4) Gonial index (GI): It is the mandibular thickness measured at the posterior border of the ramus (154).
5) Mandibular ratio (M/M): It is the ratio between the total height of the mandibular body and the height from the lower border of the mandible to the lower border of the mental foramen (155)

Amongst the qualitative morphometric parameters, the Klemetti index (KI) is by far the most widely adopted index. The KI, also known as mandibular cortical index, classifies the mandibular cortex distal to the mental foramen in three categories:

- C1, when the endosteal margin is even and sharp;
- C2, when the endosteal margin presents lacunar resorption or cortical residues on one or both sides;
- C3, when there is a clearly porous cortical layer, with heavy endosteal cortical residues (156)

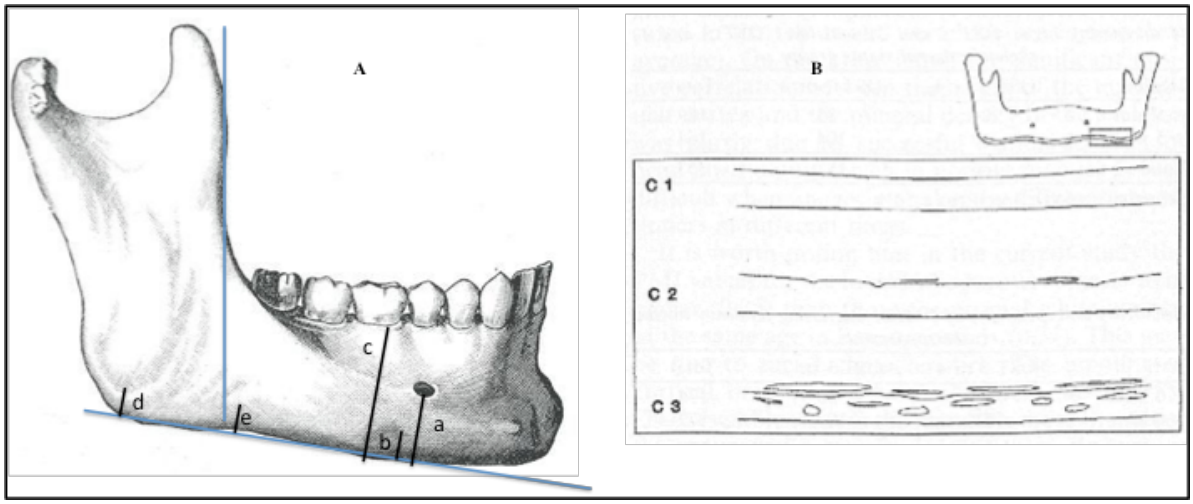

Fig. 20 A) Quantitative indices: MCW= b; PMI= b/a; GI= d; AI=e ; M/M= c/a

(B) Klemetti index (or MCI)

2 HYPOTHESIS AND AIM OF THE RESEARCH

2.1 BACKGROUND AND HYPOTHESIS

Osteoporosis is often referred to as a "silent disease," since quite often it is not diagnosed until a fragility fracture occurs. Osteoporotic fractures are expected to occur in approximately one in two Caucasian women and one in five men, thus representing a worldwide burden with an associated high morbidity and mortality. The measurement of bone mineral density (BMD) at the femur neck and lumbar spine with dual-energy X-ray absorptiometry (DXA) is the gold standard for osteoporosis diagnosis. Although there is indirect evidence that supports the screening of population groups (mainly women >65 years old) to reduce the risk of fractures by identifying and treating people with reduced BMD, in most countries the cost of a general screening programme is not affordable or cost effective. As a consequence, osteoporotic patients are often undiagnosed or untreated for long periods of time. Remarkably, only 23% of women >67 years old who have experienced an osteoporosis-related fracture receive either a BMD test or an osteoporotic medication in the six months after the fracture, as reported by the National Committee for Quality Assurance (National Committee for Quality Assurance 2014).
Considering that dental panoramic radiographs (OPGs) are frequently performed for diagnostic purposes during dental check-ups or before several dental procedures (Tugnait, Clerehugh et al. 2003), it would be of great value if they could be used in identifying patients at risk of osteoporosis. The idea is that dentists could potentially spot previously undiagnosed osteoporotic patients and refer them to the specialist, where they could be treated before developing a fracture.

Periodontitis is a chronic inflammatory disease that affects the supporting tissues of teeth (periodontal ligament, gum and bone) and, if untreated, can eventually lead to tooth loss. According to the National Health and Nutrition Examination Survey (NHANES), 46% of adults presented with periodontitis in US in 2009 to 2012, thus accounting for 64.7 million people, with 8.9% of them presenting severe periodontitis. The demonstration of a relationship between osteoporosis and periodontitis is difficult, especially in consideration of the fact that they are multifactorial diseases and that the common risk

factors may introduce additional biases. Although several cross-sectional studies have reported an association.

2.2 AIM OF THE STUDY

2.2.1 PRELIMINARY LITERATURE REVIEWS

1. To do a narrative review on the available evidence on the correlation between skeletal and jawbone density in osteoporotic subjects

2. To do a narrative review on the available evidence on the correlation between periodontitis and osteoporosis

2.2.2 PRIMARY OUTCOMES

3. To assess the feasibility of using dental panoramic indices to screen for post-menopausal osteoporosis

4. To assess the sensitivity and specificity of Klemetti Index (KI) to screen for osteoporosis in postmenopausal women

5. To assess the sensitivity and specificity of quantitative panoramic indices (Mandibular Cortical Width and Panoramic Mandibular Index) to screen for osteoporosis in postmenopausal women

6. To assess the precision and reproducibility of panoramic indices to screen for osteoporosis in postmenopausal women

2.2.3 SECONDARY OUTCOMES

7. To assess a statistical significant correlation between periodontitis and osteoporosis

3 LITERATURE REVIEWS

3.1 SYSTEMIC MINERAL BONE DENSITY AND JAW BONES

Recently, Calciolari et al (Calciolari, Donos et al. 2015) conducted a systematic review of the literature and meta-analysis on the accuracy of panoramic morphometric indices in detecting reduced BMD. 50 studies meeting the inclusion/exclusion criteria were included in the qualitative evaluation, but the risk of bias assessment (performed with the QUADAS-2 tool) revealed that a great part of the studies was not of high quality. In particular, methodological concerns were associated to the index test and patient selection domains, as several studies did not report clearly the patients' inclusion/exclusion criteria, they did not state if the examiners were blinded to the patients' skeletal BMD or they did not measure intra/inter observer agreement. 19 studies were eventually included in the meta-analysis, which could be performed only for three indices: MCW, PMI and KI. Within the limitations of this review, the authors concluded that PMI with a cut-off value of 0.3 seemed the most accurate linear index to screen for reduced BMD (sensitivity and specificity above 70%). Less strong conclusions could be drawn for MCW, but this index seemed more useful to exclude the risk of low BMD, since in 90% of the cases patients with a cortical width wider than 4 mm had a normal skeletal BMD. The qualitative evaluation of mandibular cortical erosions (KI) was also found to be a useful and reliable indicator of reduced skeletal BMD, since in approximately 80% of the cases it was associated with at least osteopenia.

As no panoramic index with 100% sensitivity and specificity has been identified yet, in some studies it has also been proposed to combine different indices between them (Klemetti, Kolmakov et al. 1994, Miranda, Arita et al. 2012) or with well-known clinical risk factors for osteoporosis (such as BMI) (Horner, Devlin et al. 2002).

Since the systematic review by Calciolari et al has included studies till March 2014, the same search strategy has been used in October 2018 to update the recent review with the literature of the last four years.

The applied research strategy included terms related to the population and the intervention investigated and has been performed in two databases, MEDLINE via OVID and EMBASE, update in October 2013.

No language restrictions have been applied. The search strategy for MEDLINE and EMBASE have used a combination of MeSH terms and text words which have been combined as Population AND Intervention. Tab. 3

MEDLINE via OVID			
	Mesh terms	**Free-text search**	**Limits**
Population	bone disease, metabolic/ or exp bone demineralization, pathologic/ or exp osteoporosis	osteoporo$ OR osteopeni$	NOT (animals NOT humans)
Intervention/Exposure	Bone Density OR exp Densitometry exp Jaw OR exp Jaw Edentulous	(bone adj2 densit$) OR (bone adj2 content) OR bmd OR bmc OR densitometr$ jaw$ OR mandib$ OR maxill$ or edentul$	
EMBASE			
	Emtree terms	**Free-text search**	**Limits**
Population	exp. osteoporosis OR osteopenia	osteoporo$ OR osteopeni$	NOT (animals NOT humans)
Intervention/Exposure	Bone Density OR Bone densitometry Jaw OR Edentulousness	(bone adj2 densit$) OR (bone adj2 content) OR bmd OR bmc OR densitometr$ jaw$ OR mandib$ OR maxill$ or edentul$	

Tab. 3 Search strategy

Due to the large volume of literature on this topic, a three-stage screening has been applied to increase the precision of screening. All stages (titles, abstract, full-text) has been carried out by the reviewer. At the stage of full-text screening, a data extraction form was completed to check eligibility of the studies and, if eligible, to collect detailed information about population, intervention and outcomes.

Seven articles have been included in the 2018 update, according to the inclusion criteria of the 2015 review by Calciolari et al. All the selected papers have described:

- Total number of patient and the partition in healthy, osteopenic and osteoporotic
- Almost one panoramic index used for the prediction of the bone mass condition

- The groups considered to be matched for the comparison
- At least one of these information: sensitivity, specificity, number of false/true negative/positive, ROC curve.

The indices that have been used most frequently to screen for reduced BMD have been:

Mandibular cortical width (MCW): In the original revision, 34 studies reported measurements of the width of the mandibular cortex, which was referred to as the mandibular cortical width (MCW) or mental index (MI) or mandibular cortical thickness (MCT). In most of the studies, MCW was measured in the mental foramen region, along a line passing through the middle of the mental foramen and perpendicular to the tangent to the lower border of the mandible either manually or digitally, with image analysis systems. The cutoff values were chosen after drawing the ROC curve in order to find the highest sensitivity/ specificity and they ranged from 2.69 mm to 5 mm. The levels of sensitivity and specificity associated with this index were heterogeneous and had a reciprocal relationship that varied in relation to the threshold chosen. A few studies reported a sensitivity >95% (157, 158), while in other studies, this parameter did not reach 20% (157, 159). The same variability applied to the specificity levels. The update, has shown that in the articles published in the last four years, the analysis of the width of the mandibular cortex has been reported in all the articles. The cutoff values were chosen after drawing the ROC and ranged from 1.5 mm to 4 mm. Similarly to the 2015 data, sensitivity and specificity were heterogeneous. Carmo et al. (160) have reported a sensitivity/specificity >95%, but other studies (161-164) have shown levels < 70%.

Panoramic mandibular index (PMI): In the original revision, 9 studies considered the panoramic mandibular index (PMI). This index represents the ratio between the mandibular cortical width at the mental foramen region and the distance from the lower border to the inferior edge of the mental foramen (152). Most of the studies reported a cutoff value of 0.3, with levels of sensitivity and specificity in detecting individuals with reduced bone density (T score <–1) ranging from 40.8% to 100% and from 47% to 88%,

respectively. Only 1 study considered 4 different cutoffs (159). Only one studi by Balto et al. has considered the PMI as predictor of osteoporosis, reporting a AUC of 0,591 (161).

Klemetti index (KI): Twenty-seven studies considered the Klemetti index (KI) as a tool for prediction of reduced BMD according to Calciolari et al.(165) This index, also known as the mandibular cortical index, qualitatively classifies the mandibular cortex distally to the mental foramen in the following categories: C1, when the endosteal margin is even and sharp; C2, when the endosteal margin presents lacunar resorption or cortical residues on one or both sides; and C3, when the cortical layer is clearly porous, with heavy endosteal cortical residues. In the included studies, the presence of cortical erosions (either C2 or C3 type) produced a sensitivity in detecting reduced BMD (T score <–1) ranging from 48.7% to 100% and a specificity ranging from 31% to 88.89%. The sensitivity in the diagnosis of osteoporosis (T score ≤–2.5) varied from 35.9% to 90.9% and the specificity from 7.8% to 93.9%. The wide range of outcomes reported is a sign of the high heterogeneity between the studies. Only a few studies evaluated the accuracy of clearly eroded cortex (C3) in detecting osteoporosis (151, 166, 167). Only one of the recently published articles has reported data on klemetti index. In the paper by Carmo et al. the presence of cortical erosions (either C2 or C3 type) produced a sensitivity in detecting reduced BMD (T score <–1) of 100% and a specificity of 90,1%. The sensitivity in the diagnosis of osteoporosis (T score ≤–2.5) was 95% and the specificity was 98,9% (160).**Tab. 4**

Author (year)	Total N. pts	N. Ost	N. Osp	N. Healthy	Age (y±SD)	Panoramic index	Type measurement	Cutoff	Groups matching	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	AUC (SE, 95% CI)
Balto et al. (2018)	431	75	124	232	57.78± 6.24	MCW	Manual annotation	4.6 mm	Healthy vs. osp+ost	58.4	60.2			0.620 (0.031, 0.550-0.690)
								4.1 mm	Healthy +osp vs. ost	69.4	68.4			
						PMI	Manual annotation							0.591 (0.036, 0.521-0.662)
						M/M	Manual annotation							0.592 (0.041, 0.512-0.672)
Carmo et al. (2017)	198W	20 (lumbar spine)	101 (lumbar spine)	77 (lumbar spine)	53.1±5.0	KI	Visual	C1 vs. C2+C3	Healthy vs. osp+ost	100%	90.1%	94.5%	100%	
								C1+C2 vs. C3	Healthy +osp vs. ost	95%	98.9%	90.5%	99.4%	
						MCW	Manual annotation	3 mm	Abnorma vs. normal *	100%	67.5%	82.9%	100%	
		11 (femoral neck)	77 (femoral neck)	110 (femoral neck)		KI	Visual	C1 vs. C2+C3	Healthy vs. osp+ost	100%	63.6%	68.8%	100%	
								C1+C2 vs. C3	Healthy +osp vs. ost	100%	94.7%	52.4%	100%	

						MCW	Manual annotation	3 mm	Abnormal vs. normal *	100%	47.3%	60.3%	100%	
Kathirvelu et al. (2014)	64W	36	28		52.5±12.7	MCW	Manual annotation	unclear	Healthy +osp vs. ost					0.856 (0.05, 0.759-0.953)
Papamanthos et al. (2014)	315w	106	103	106	59.64±8.19	MCW	Manual annotation	Osteopenic</=3.69	Healthy vs osteopenic	66.99	62.26			0.656
								Osteoporotic </=3.24	Healthy vs osteoporotic	80.19	81.13			0.872
								Osteoporotic</=3.24	Osteopenic vs osteoporotic	80.19	72.82			0.809
Anburajan (2014)	141W	21 (spine)/20 (femoral neck)	120 (spine)/121 (femoral neck)		49.07±3.06	MCW	Computer-aided system	unclear	Healthy vs. ost					0.886 (0.03, 0.840-0.941)
Nagi et al. (2014)	120w	60	0	60	60.17 ± 5.5	MCW	Manual annotation	3.35	Healthy vs osteoporotic	55	93.3			0.778 (0.693 – 0.849)

Kim et al. (2014)	194W	Unclear	Unclean	Unclear	65.6±8.6	MCW	Computer-aided system	1.5 mm	Healthy +osp vs. ost	14.3	96.2			0.737
								2 mm		35.7	91.8			
								2,22 mm		67.9	78.5			
								2.5 mm		67.9	69.6			
								3 mm		85.7	43.7			
								3.5 mm		67.9	78.5			

Tab. 4 Papers included in the review (2014-2018)

3.2 PERIODONTITIS AND OSTEOPOROSIS

Despite the investigation on the relationship between the incidence of periodontitis in osteoporotic patients has been considered a secondary outcome, a narrative review has been carried out to summarize the controversial available literature about the correlation and to better understand the position of the international scientific community.

The question addressed was the following: "Is periodontitis correlated with osteoporosis?" and the applied research strategy included terms related to the population and the intervention investigated and has been performed in two databases, MEDLINE via OVID and EMBASE, update in 2018.

No language restrictions have been applied. The search strategy for MEDLINE and EMBASE have used a combination of MeSH terms and text words which have been combined as Population AND Intervention. Tab. 5

MEDLINE via OVID / EMBASE			
	MeSH terms	**Free text**	**Limits**
Population	Bone disease, metabolic/or exp bone demineralization, pathologic/or exp osteoporosis	Osteoporo$ OR osteopeni$ OR (bone loss adj2 age related)	NOT (animals NOT humans)
Intervention/Exposure	Exp Periodontal Diseases/	Periodont$ or parodontos$ or pyorrhea or furcation	

Tab. 5 Search strategy

The studies assessing the correlation between periodontitis and osteoporosis have been considered. Only studies with at least five patients were selected to exclude individual case reports. Studies carried out as case-control or cohort or cross-sectional have been included. Studies have been included if they directly compared the association between osteoporosis and chronic periodontitis, measured in terms of clinical attachment level

(CAL) and/or pocket probing depth (PPD). The considered diagnosis of osteoporosis has been the DXA test at either hip or spine.

Articles published from 1949 to 2018 have been considered. Interestingly, the number of articles related to this topic has progressively increased in the past years. Fig. 21

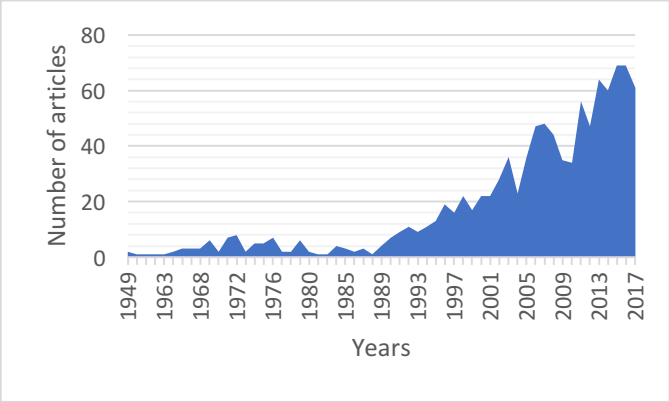

Fig. 21 Number of article per year from 1949 to 2018

A three-stage screening (titles, abstract, full-text) was applied to increase the precision of the review. At the stage of full text screening, a data extraction form was completed to check the eligibility of the studies and to collect detailed information about population, intervention and outcomes. Tab. 6

Fifteen studies have matched with the inclusion criteria for the literary review.

Pilgram et al. have found a weak positive correlation between the periodontal status and BMD in longitudinal follow-up of 3 years though no correlation could be seen at cross-sectional level in 135 participants (168). Brennan has found a significant correlation between CAL and BMD of total forearm, worst site T-score, anteroposterior spine, and whole body (119) while Tejal has reported a weak correlation between the CAL and systemic osteoporosis (r = 0.10–0.17), which has not reached a statistical significance .

Although Weyant has found no statistically significant association between periodontal indicators and BMD in 292 participants, a trend toward a more severe periodontal disease was seen in all their measurements with decreasing BMD, indication of a possible

association (117). Moeintaghavi and Marjanovic also have not found any statistically significant association between osteoporosis and periodontitis, but the sample size has been very small (169, 170)

Ten out of 15 studies included have reported a significant association between periodontitis and osteoporosis. Most of the included studies have been either cross sectional or prospective studies and included post-menopausal women. The majority of the studies have reported values of CAL and have used the correlation or regression coefficients or odd ratios.

In conclusion, the literature points out that could be a correlation between oral bone loss and skeletal osteoporosis but the topic is still controversial. Several studies have also reported a correlation between systemic osteoporosis and number of missing teeth, reduced alveolar crestal height, or tooth mobility. When tooth loss occurs, there is no mechanical stress in its antagonistic and in the alveolar bone supporting. The consequence is the expansion of the bone marrow cavity together with a loss in bone volume.

For all these reasons, the association between these two diseases is biologically plausible and several mechanisms have been proposed to link them. It can be speculated that the reduced BMD associated with osteoporosis might also affect the jawbones and might accelerate alveolar bone loss following insult by periodontal bacteria (118).

Author (year)	Total N. pts	Age (y±SD))	Periodontal disease cut-off	Bone Mineral Density	Number of patients with osteoporosis	Number of patients with periodontitis	Association between CAL and osteoporosis
Brennan et al. (2007)	1329	66.6±7.0	CAL	DEXA spine, hip, forearm, whole body	Normal 508 (44.8%) Osteopenia 469 (41.4%) Osteoporosis 157		yes
Passos et al. (2013)	521	60.8	PPD >5 mm CAL >6 mm	DEXA proximal Femur and lumbar spine	Osteoporosis 380 Osteopenia 141	94	yes
Habashneh et al. (2010)	400	62.5±6.4	PPD, CAL 5 mm PD/6 mm CAL	DEXA lumbar spine (L1-L4) and femoral neck	Normal 94 (23.5%) Osteopenia 170 (42.5%)		yes

					Osteoporosis 136 (34.0%)		
Iwasaki et al. (2013)	397	68.2 (60-80)	CAL, PPD ≥4 mm	DEXA lumbar spine (L2-L4), fel neck	Normal 161 (44.8%) Osteopenia 136 (41.4%) Osteoporosis 100	142 (35.8%)	Yes
Marjanovic et al. (2013)	380	45-65	PPD >5.5 mm CAL >7 mm	DEXA proximal Femur and lumbar spine	Osteoporotic 98 Non osteoporotic 282	150	No
Weyant et al. (1999)	292	75.5±4.38	PPD, CAL	DEXA proximal Femur and lumbar spine		142	No
Gondim (2013)	148	58.93±4.7	CAL, PPD Moderate: CAL ≤5 mm Severe: CAL >5 mm	DEXA lumbar spine (L1-L4), femoral neck, and total femur			Yes
Gomes-Filho et al. (2007)	139	58.8±6.4	PPD >4 mm CAL >3 mm	DEXA femur/spine	40 cases 60 controls	48	Yes
Pilgram et al. (2002)	135	59	PPD CAL	DEXA proximal Femur and lumbar spine			No
Penoni et al. (2015)	134	69.84	PPD >5 mm CAL >6 mm	DEXA proximal Femur, total femur and lumbar spine	Normal 48 Osteoporosis 86	51	Yes
Juluri et al. (2015)	100	60.12	CAL PPD	DEXA lumbar spine	50 cases 50 controls		Yes
Singh et al. (2014)	78		PPD, CAL	DEXA lumbar spine (L1-L4)	Normal 22 Osteopenia 25 Osteoporosis 31		Yes
Tejal et al. (2000)	70	62.10±7.1	CAL, ABL	DEXA proximal Femur and lumbar spine			No
Moeintaghavi et al. (2013)	60	53.05	PPD, CAL	DEXA proximal Femur and lumbar spine	Normal 20 Osteopenia 20 Osteoporosis 20		No
Grocholewicz et al. (2012)	37	59.4±5.6	PDI of 6 indicates an attachment loss of ≥6 mm	DEXA lumbar spine (L2-L4), femoral neck, and distal radius			Yes

Tab. 6 Papers included in the review

4 CLINICAL STUDY DESIGN

In the past years, specific quantitative and qualitative indices/parameters, which can be calculated on dental panoramic radiographs, have been proposed as tools to detect osteoporosis, with different levels of accuracy.

Amongst the quantitative indices, the most adopted ones are the mandibular cortical width (MCW) and the panoramic mandibular index (PMI).

Regarding the qualitative indices, the Klemetti index (KI) is by far the most applied one.

A recent systematic review and meta-analysis from our group (165) showed that the presence of any kind of cortical porosity (C2+C3) is associated with a sensitivity and specificity in detecting osteoporosis of 80.6% and 64.3%, respectively. The advantage of using this index, compared to others available, is that it is straightforward and relatively easy to measure and it does not require specific softwares.

It is clear that the panoramic indices cannot replace the diagnosis of osteoporosis by BMD measurement with DXA scan. However, whenever a panoramic radiograph is available, they might be opportunistically used to detect previously undiagnosed osteoporotic patients and refer them to a specialist.

While several studies support the use of panoramic indices, they have never been tested in an Italian University Hospital setting and in a study adequately powered and controlled for confounding variables and therefore able to put together the information of the KI with DXA.

4.1 OVERALL STUDY PLAN

This is cross-sectional observational study aiming to recruit a cohort of 124 consecutive post-menopausal women. The only study visit took place at the "Centro Universitario di Odontoiatria, Dipartimento di Medicina e Chirurgia, Università di Parma".

All post-menopausal women ≥ 65 years old attending the Centro Universitario di Odontoiatria in the new patients' or follow-ups' clinics were approached to check for the inclusion/exclusion criteria and their willingness to take part in the study. Furthermore, doctors (general practitioners or osteoporosis specialists) were contacted and will be kindly asked to inform their patient that had done a DXA scan within the past 12 months

about the study. Potentially eligible patients were booked an appointment at the "Centro Universitario di Odontoiatria, Dipartimento di Medicina e Chirurgia, Università di Parma" to check for inclusion/exclusion criteria and for the recruitment. As part of the study, they received a full-mouth examination of the hard and soft tissues and an OPG at no cost. Before enrolment, written and verbal information was given to the patients and written informed consent was obtained.

4.2 INCLUSION CRITERIA

Each participant had to meet the following inclusion criteria to be enrolled in the study:

- ≥ 65 years old
- In self-reported menopause, defined as the permanent cessation of ovulation, for at least one year before the visit (171).
- A DXA examination at the hip and lumbar spine performed within the previous 12 months

4.3 EXCLUSION CRITERIA

Participants were not eligible for participation in the study if:

- Affected by systemic diseases (with the exception of osteoporosis) recognized to severely affect bone metabolism (e.g. Cushing's syndrome, Addison's disease, diabetes mellitus type 1, leukaemia, pernicious anaemia, malabsorption syndromes, chronic liver disease, rheumatoid arthritis).
- Knowingly affected by HIV or viral hepatitis.
- History of local radiation therapy in the last five years.
- Affected by limited mental capacity or language skills such that study information cannot be understood, informed consent cannot be obtained, or simple instructions cannot be followed.

- Other severe acute or chronic medical or psychiatric condition or laboratory abnormality which may increase the risk associated with trial participation or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the participant inappropriate for entry into this trial.

4.4 STUDY VISITS

Only 1 study visit was performed with data collection:

- Signing of informed consent;
- Recording of any concomitant medication;
- Confirmation of participant eligibility in relation to the inclusion/exclusion criteria;
- Examination of hard and soft tissues;
- Full-mouth six-points periodontal chart, with recording of probing pocket depth (PPD), recession (REC), furcation involvement and mobility.
- Dental panoramic radiograph (OPG) if not performed at the Centro Universitario di Odontoiatria within the previous 12 months

4.5 STUDY MEASUREMENTS

4.5.1 Dental panoramic radiographs

If not performed within the previous 12 months at the Centro Universitario di Odontoiatria, an OPG was performed during enrolment visit.

OPGs are routine low-dose x-rays that are performed during dental check-ups, follow-ups or before dental treatments (e.g. extractions, implant placement, etc.), as they allow the evaluation of bone and dental structures of both jaws, the sinus and temporo-

mandibular joints. They are routinely done to each new patient attending the Centro Universitario di Odontoiatria.

An ORTHOPHOS XG 3D (Sirona Dental) machine will be used and the standard settings recommended by the developer will be applied.

Two examiners blinded to the DXA results (provided by the patients) independently measured the panoramic indices on the OPGs. In particular, for the KI, the level of mandibular cortical erosion in the mental foramen region was qualitatively evaluated after zooming the OPG and optimization of the image contrast and white balance.

The MCW and PMI were measured with the digital software provided by ORTHOPHOS XG 3D.

After at least 1 week, the examiners will repeat the measurements in 20% of the OPGs, in order to evaluate intra-examiner reproducibility.

4.5.2 Periodontal Examinations

A complete medical and dental history were obtained during enrolment visit. The histories included demographic background information, systemic and dental status information.

During the enrolment visit, a detailed examination of the hard and soft tissues was performed and a trained examiner also registered the probing pocket depth (PPD), gingival recession (REC), at six sites (mid-buccal, buccal, disto-buccal, lingual, mid-lingual and disto-lingual) around each tooth with a UNC-15 probe. Furcation involvement was assessed with a Nabers probe and mobility was assessed with the help of a mirror.

The results of the dental and periodontal examination were explained to the patients.

It is important to highlight that the periodontal and dental examination were performed as part of the standard examination that each patient receives when attending the Centro Universitario di Odontoiatria or any other dental centre.

4.6 STATISTICAL PROCEDURES

4.6.1 Sample size estimation

The sample size was based on the results of sensitivity and specificity of the KI (primary outcome) obtained from a systematic review and meta-analysis recently published by our group (Calciolari et al. 2015) and on the level of sensitivity of a panoramic index that both osteoporosis experts and patients would consider as meaningful. In fact, during a Patient and Public Involvement (PPI) group, both the patients and the consultants interviewed agreed that a test that correctly screen for osteoporosis in 80% of the cases would be worth, provided that the lower confidence limit is at least 55%. By taking into consideration these target values, and speculating a prevalence of osteoporosis of 30% amongst post-menopausal women attending a dental hospital, 124 participants needed to be recruited for this study.

4.6.2 Statistical method

The following data were recorded:

- Sensitivity, specificity, false positive rate, negative positive rate of Klemetti Index (primary outcome) in detecting osteoporosis. The presence of a high level of mandibular cortical erosion (C3) were considered as a sign of osteoporosis, while a sharp and even endosteal margin (C1, C2) was considered a sign of normal bone mineral density.
- Sensitivity, specificity, false positive rate, negative positive rate and ROC curve of Mandibular Cortical Width and Panoramic Mandibular Index in detecting osteoporosis
- Statistical significant correlation between periodontal status and reduced systemic bone density (Periodontal probing depth (PPD), Full Mouth Bleeding Score (FMBS), Full Mouth Plaque Score (FMPS), Clinical attachment loss (CAL), Recession (REC), tooth mobility (MOB), furcation involvement (FURC), tooth loss).

SPSS software package has been used.

5 RESULTS

5.1 GENERAL AND DEMOGRAPHIC DATA

At the moment of the drawing up of this thesis, patient's enrolment is still in progress. 70 patients have been enrolled to take part to the clinical study. All the patients have satisfied inclusion criteria:

- ≥ 65 years old female
- In self-reported menopause
- With a DXA examination at the hip and lumbar spine performed within the previous 12 months

During the study visit, age, ethnicity, smoke habits, body mass index, height and medications have been recorded in the Clinical Report Form (CRF). Tab. 16

5.2 OPG INDICES AND OSTEOPOROSIS DATA

Dxa scan results have been registered in the Clinical Report Form (CRF) and patients have been classified in healthy, osteopenic and osteoporotic according to the T-score of either lumbar spine or femur neck. The worst value has been used to classify the bone density status. Tab. 17

The distribution of the t-score values between the sample has been:

- Healthy patients: 2 (3 %)
- Osteopenic patients: 31 (44,2 %)
- Osteoporotic patients: 37 (52,8)

According to the literature, patients have been classified into two main groups:

- healthy + osteopenic (0): 33 (47 %)

- Osteoporotic patients (1): 37 (53 %)

OPGs have been analysed to obtain morphometric indices values such as Klemetti index (KI), Mandibular Cortical Width (MCW), Mental index (MI), Panoramic mandibular index (PMI), Antegonial index (AI), Gonial index (GI) and Mandibular ratio (M/M). Tab. 18

All the patients have been analysed for the Klemetti index and the distribution between the sample has been:

- C1: 13 (18 %)
- C2: 28 (40,5 %)
- C3: 29 (41,5 %)

According to the literature, KI has been classified into two main groups:

- C1 + C2 (considered healthy): 46 (58,5 %)
- C3 (considered osteoporotic): 29 (41,5 %)

Since the Klemetti clusters can be used to quantitatively classify healthy, osteopenic and osteoporotic patients, sensitivity and specificity have been calculated considering the Klemetti index as a tool for prediction of reduced BMD (T-score</= -2,5). The presence of cortical erosion (C3 vs. C1-C2) has produced a sensitivity of 61,1 % and a specificity of 79,4 % with a positive predictive value of 75,9 % and a negative predictive value of 65,9 %.

KI	
True positive (TP)	22
True negative (TN)	27
False positive (FP)	7
False negative (FN)	14
Sensitivity	61,1 %
Specificity	79,4 %
Positive predictive value	75,9 %
Negative predictive value	65,9 %

Tab. 7 Klemetti index accuracy

Mandibular cortical width (MCW) index has been analysed on 51 patients because of some technical issues in detecting the required measurements with accuracy in the remaining 19 patients. The cutoff value has been chosen after drawing the ROC curve in order to find the highest sensitivity/specificity and it has been set at 3,33 mm. The levels of sensitivity and specificity associated with this index have been 72,2 % and 64,7 % respectively. Tab. 8 - Tab. 9 - Fig. 22

MCW				
Area	Standard error	Asintotic significance	Asintotic Confidence Interval 95%	
			Inferior limit	Upper limit
0,676	0,081	0,038	0,517	0,836

Tab. 8 MCW ROC - area under the curve data

MCW	
Sensitivity	72,2 %
Specificity	64,7 %

Tab. 9 Mandibular cortical width index accuracy

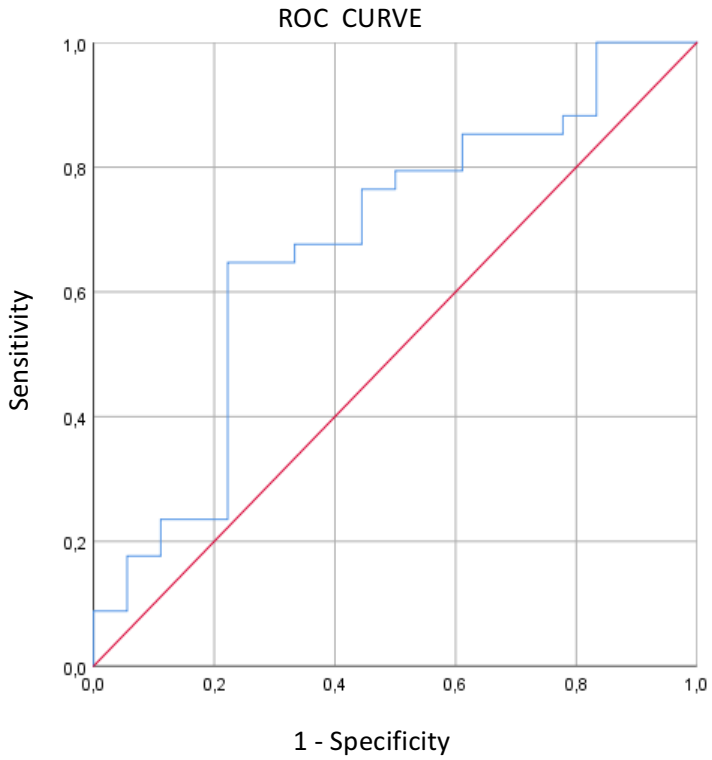

Fig. 22 Mandibular cortical width ROC curve

A correlation between Klemetti Index and Mandibular Cortical Width has been investigated for the group of 51 patients. A Mann-Whitney test has been applied on the results obtained by the same population and a statistical significative correlation between MCW and the Klemetti C1+C2 vs. C3 has been found (p=0,001).

According to this finding, the KI value for healthy patients has been correlated to patients with a MCW higher than the cut-off value as a combined tool to predict osteoporosis. After this analysis, sensitivity and specificity have been calculated for the combined indices and the result has been 88,8 % and 58,8 % respectively, with a negative predictive value of 90,9 %. Tab. 10

MCW/KI	
Sensitivity	88,8 %
Specificity	58,8 %
Negative predictive value	90 ,9 %

Tab. 10 Accuracy of KI in association with MCW when related to t-scores

Any other combination of MCW and KI (KI=C3 and/or MCW<3,33 mm) have been statistically tested but no improvement in sensibility, specificity or predictive values have been observed.

Panoramic Mandibular Index (PMI), Mental ratio (M/M), antegonial index (AI) and gonial index (GI), have been also tested as predictor tools in detecting osteoporosis. A ROC curve has been drawn and the area under the curve has been identified. Low level of sensitivity and specificity have been reported for all these indices. Tab. 11 - Fig. 23

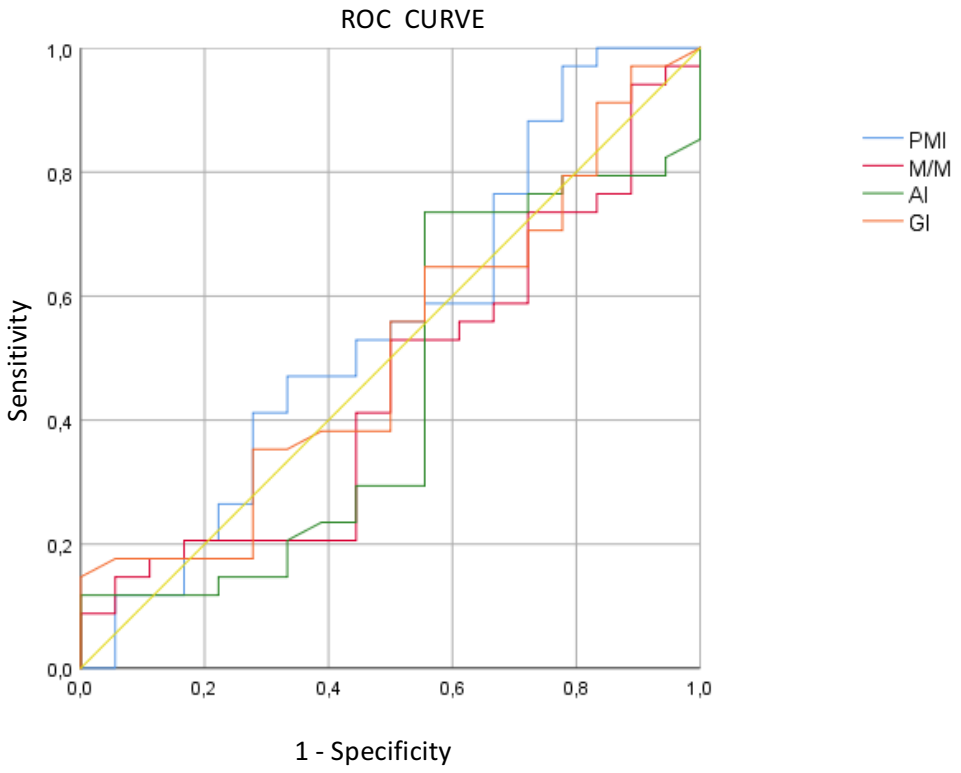

Fig. 23 PMI, M/M, AI, GI ROC curve

SECONDARY INDICES					
index	Area	Standard error	Asintotic significance	Asintotic Confidence Interval 95%	
				Inferior limit	Upper limit
PMI	0,552	0,088	0,538	0,380	0,725
M/M	0,456	0,085	0,604	0,290	0,622
AI	0,444	0,087	0,513	0,273	0,616
GI	0,512	0,085	0,885	0,346	0,678

Tab. 11 Accuracy of panoramic mandibular index, Mental ratio, antegonial index and gonial index

5.3 PERIODONTAL DATA

Periodontal data such as Periodontal probing depth (PPD), Full Mouth Bleeding Score (FMBS), Full Mouth Plaque Score (FMPS), Clinical attachment loss (CAL), Recession (REC), tooth mobility (MOB), furcation involvement (FURC) have been registered in a complete periodontal chart (Tab. 19). Both complete edentulous patients or dentate patients have been enrolled for the study and the number and percentage of missing teeth have been taken into account. According to the literature, only patients with at least 8 teeth have been considered dentate patients and included in periodontal data analysis. Fifty-eight dentate patients have been classified according to t-score cut-off. The distribution within the sample has been 24 patients for the healthy group and 34 patients for the osteoporotic group. A statistical significance has been investigated between periodontal indices and diagnosis of osteoporosis according to the T-score (0= healthy/osteopenic; 1=osteoporotic). A Kolmogorov-Smirnov test has been performed to assess the normality of the distribution of the periodontal data samples. The K-S test has identified the clinical attachment level as a normal distribution variable. In this case a t-test has been applied to investigate the statistically significant correlation in case of normal distribution

(average and standard deviation). In case of PPD, REC, MOB and FURC a Mann-Whitney test has been used to test the correlation (median and quartile).

Periodontal probing depth (PPD): 58 patients have been considered for the statistical analysis. A six-sites periodontal probing has been performed on each tooth and the probing depth has been classified in two clusters according to a cut-off of 5 mm.
Since the pocket probing depth has been defined as a non-normal distributed variable (Kolmogorov-Smirnov test), median and 25 % quartile have been calculated. A statistically significant correlation between PPD and BMD has been found for the patients with PPD>/= 5mm (p=0,006). The median of the patient with PPD >/= 5 mm group has been 9,75 % (25 quartile: 2,7 %) in the healthy group, and 3,45 % (25 quartile: 0,6 %). Tab. 12

Sample	N	Median (%)	25 quartile (%)
Healthy	24	9,75	2,7
Osteoporotic	34	3,45	0,6

Tab. 12 Pocket probing depth (PPD) statistical analysis: median and 1st quartile

The aforementioned correlation has been tested between PPD and BMD, considered as a dichotomic value defined on the t-score cut-offs. The correlation has been also tested considering t-score as a continuous variable and a Spearman's rank correlation coefficient has been applied. Spearman's rho has shown a statistical significance (p=0,005). Fig. 24

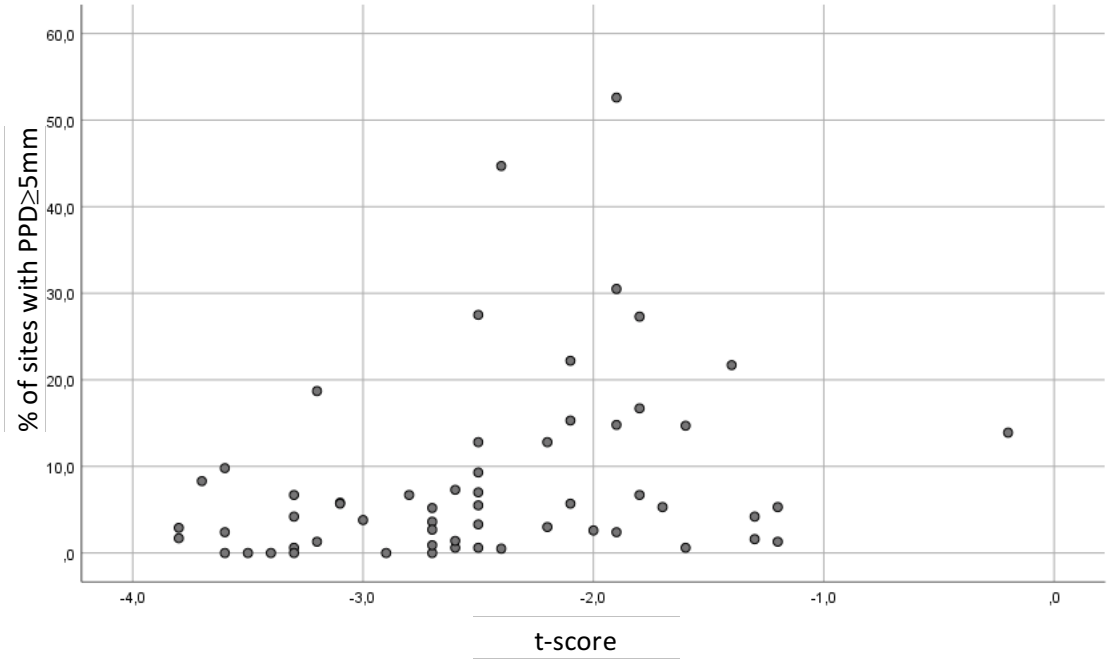

Fig. 24 Scatter plot representing the distribution of the sites with a PPD≥ 5 mm in respect of t-scores as a continuous variable

Clinical attachment loss (CAL): Clinical attachment loss has been defined as the REC in addition to PPD values. Average and standard deviation have been calculated and t-test has been performed to demonstrate a correlation between CAL and t-scores. Sites have been classified in three clusters, with CAL of 1-2 mm, CAL of 3-4 mm and CAL > 5 mm. Only the cluster of 3-4 mm of CAL has shown a statistically significant correlation with t-scores classification (healthy and osteoporotic) with a p=0,21. The average of sites with CAL of 3-4 mm has been 49,4 % (SD 11,05 %) for the healthy group and 56,9 % (SD 12,6 %) for the osteoporotic group.Tab. 13

Sample	N	CAL groups	Average (%)	Standard deviation (%)
Healthy	24	% sites with CAL 1-2 mm	22,429	14,8368
		% sites with CAL 3-4 mm	49,417	11,0525
		% sites with CAL > 5 mm	28,563	20,2506
Osteoporotic	34	% sites with CAL 1-2 mm	22,615	13,6314
		% sites with CAL 3-4 mm	56,982	12,6118
		% sites with CAL > 5 mm	20,388	15,1708

Tab. 13 Clinical attachment loss (CAL) statistical analysis: average and standard deviation

The Spearman's rank correlation coefficient has been also applied to the CAL in order to investigate the correlation between attachment level and t-score as a continuous variable. Similarly to the t-test, Spearman's rho has identified a correlation to the group with CAL of 3-4 mm (p=0,13). Fig. 25

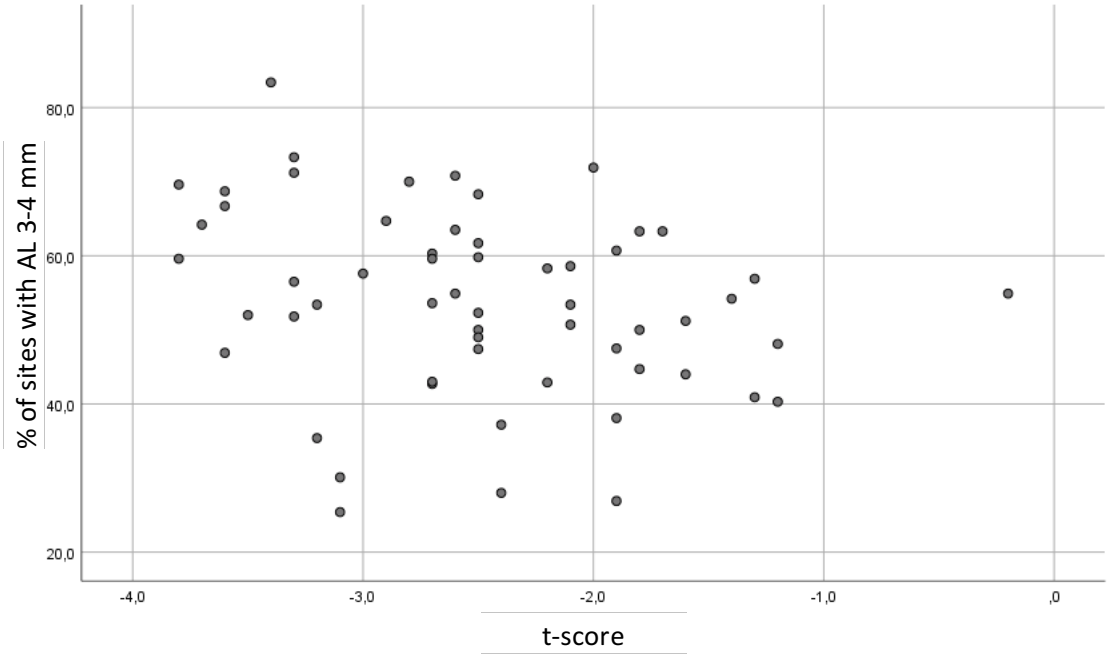

Fig. 25 Scatter plot representing the distribution of the sites with a AL 3-4 mm in respect of t-scores as a continuous variable

Tooth loss: The number of teeth loss has been analysed on the whole sample. A Mann-Whitney test for the non-parametric variables has been applied to investigate a statistical significance in relation to the t-score (dichotomic value) and a p value of 0,007 has been found. The median of the percentage of teeth loss (considered 28 teeth as 100 %) has been 25 % (25 quartile of 17,8 %) for healthy group (33 patients), and 14,3 % (25 quartile of 7,1 %) for the osteoporotic group (37 patients). Tab. 14

The Spearman's rank correlation coefficient has been also applied and no statistical differences have been demonstrated considering t-score as a continuous variable

Sample	N	Median (%)	25 quartile (%)
Healthy	33	25	17,8
Osteoporotic	37	14,3	7,1

Tab. 14 Tooth loss statistical analysis: median and 1st quartile

Recession, Furcation, Mobility: All the remaining periodontal indices that have been statistically tested, have shown no significance between groups. For the furcation involvement, the frequency has been analysed but the chi-square test has shown no significance. Tab. 15

Indices	Median (%)	25 quartile (%)
REC > 3 mm (% sites)	0,25	0
REC ≤ 3mm (% sites)	99,4	95,35
Mobility (% teeth)	6,3	0

Tab. 15 Recession (REC) and tooth mobility: median and 1st quartile

Patients ID	AGE	ETHNICITY	SMOKE	HEIGHT	WEIGHT	BMI	Vit. D	BISPHOSPHONATES
							(0= no; 1= yes)	
L-Z 001	73	caucasian	0	154,5	49	20,53	1	1
G-P 002	72	caucasian	0	165	101	37,1	0	0
M-M 003	79	caucasian	1	158,1	50	20	0	1
A-B 004	73	caucasian	1	162,5	65	24,62	0	0
R-L 005	81	caucasian	0	161	56	21,6	0	0
C-G 006	79	caucasian	0	158	74	29,64	1	0
L-M 007	68	caucasian	0	154	59	24,88	0	0
T-C 008	67	caucasian	1	157,4	59	23,81	0	0
M-G 009	68	caucasian	0	165	83	30,49	0	0
S-M 010	76	caucasian	0	157	61	24,75	0	0
O-C 011	73	caucasian	0	160,4	49	19,05	1	0
M-P 012	73	caucasian	0	156,3	53	21,69	0	0
O-C 013	74	caucasian	0	160	59	23,05	1	0
T-C 014	70	caucasian	0	160,2	60	23,38	0	0
L-B 015	67	caucasian	0	156	61	25,07	0	0
G-M 016	77	caucasian	0	153	56	23,92	1	0 (2y)
N-G 017	81	caucasian	0	144,4	57	27,34	0	0
A-B 018	66	caucasian	1	153,6	55	23,31	1	0
G-P 019	73	caucasian	0	164	56	20,82	0	0
R-G 020	80	caucasian	0	167	66	23,67	1	0
D-N 021	69	caucasian	0	153,2	48	20,45	0	0
A-C 022	72	caucasian	1	154,5	54	22,62	0	0
M-M 023	67	caucasian	0	153	62	26,49	0	0
M-S 024	69	caucasian	0	154,8	55	22,95	1	0
S-G 025	72	caucasian	0	151,2	66	28,87	0	0
L-T 026	71	caucasian	1	155,2	50	20,76	1	0
M-C 027	74	caucasian	1	162,7	57	21,53	0	0
E-S 028	76	caucasian	0	160	46	17,97	1	0
M-G 029	79	caucasian	0	152	54	23,37	0	0
A-C 030	67	caucasian	1	154	53	22,35	0	0
M-F 031	65	caucasian		162	75	28,6	1	0 (3 m)

M-M 032	65	caucasian	0	166	51	18,5	1	0
M-P 033	67	caucasian	0	148	53	24,2	1	0 (7y)
MR-B 034	65	caucasian	0	162	60	23	0	0
G-C 035	65	caucasian	0	161	57	22	1	0
A-G 036	66	caucasian	0	164	58	21,6	1	0 (5y)
G-S 037	66	caucasian	0	159	56	22,2	0	0
R-S 038	66	caucasian	0	158	89	35,9	1	1
G-S 039	66	caucasian	0	155	47	19,7	0	0
G-C 040	66	caucasian	0	146	60	27,9	1	1
MN-T 041	67	caucasian	0	160	69	27	1	0
F-D 042	66	caucasian	1	152,7	54	23,16	0	0
M-A 043	75	caucasian	0	154,7	55	22,98	0	0
MP-G 044	84	caucasian	0	159,5	66	25,94	0	0
A-D 045	67	caucasian	0	148	72	32,9	1	0
R-C 046	65	caucasian	1	156	55	22,6	1	0
L-R 047	65	caucasian	0	163	60	22,6	1	0
D-B 048	67	caucasian	0	158	54	21,6	1	0
C-P 049	67	caucasian	0	153	66	28,2	1	1
A-P 050	78	caucasian	0	160,3	63	24,52	0	0
M-B 051	74	caucasian	0	160,5	64	24,84	0	0
M-M 052	66	caucasian	0	167	103	36,9	1	0
PKS 053	60	caucasian	0	149,5	57,2		1	1
AMF 054	63	caucasian	0	152,3	65		1	0 (6m)
K-D 055	63	caucasian	0	157,6	56		1	0
C-M 056	66	caucasian	0	160	108		1	1
PFF 057	59	caucasian	0	156	60		0	0
G-G 058	75	caucasian	0	158,2	92		0	0
NMF 059	66	caucasian	0	166	62		0	1
DJG 060	69	caucasian	0	153	51		1	1
DIG 061	72	caucasian	0	155	52		0	0
LMK 062	60	caucasian	0	166	48,3	17,5	1	1
J-W 063	55	caucasian	0	162	63		1	0
J-B 064	68	caucasian	0	153	48,3		1	0
MBM 065	61	caucasian	0	165	68		1	0 (4y)

V-F 066	63	caucasian	0	161,5	52,1		1	1
SAR 067	53	caucasian	0	159	71		0	0
JAL 068	60	caucasian	0	155	52		1	0 (2y)
A-S 069	60	caucasian	0	160	58		0	no
A-L 070	70	caucasian	0	152	51,3		0	1
E-M 071	66	caucasian	1	175	85	27,8	0	0

Tab. 16 Demographic data collection. Age, etnicity, smoke, height, weight, bmi and medications have been recorded

Patients ID	Dxa scan T-score			diagnosis	classification
	lumbar spine	femur neck	worst value		
L-Z 001	-1,9	-3,4	-3,4	Osteoporotic	1
G-P 002	4,2	-0,2	-0,2	Healty	0
M-M 003	-1,6	-1,8	-1,8	Osteopenic	0
A-B 004	0,2	-2,4	-2,4	Osteopenic	0
R-L 005	-2,4	-1,4	-2,4	Osteopenic	0
C-G 006	-2,4	-2,2	-2,4	Osteopenic	0
L-M 007	-1,2	-0,7	-1,2	Osteopenic	0
T-C 008	-3,2	-3,2	-3,2	Osteoporotic	1
M-G 009	-1,2	-1,1	-1,2	Osteopenic	0
S-M 010	-3,1	-2,5	-3,1	Osteoporotic	1
O-C 011	-1	-2,7	-2,7	Osteoporotic	1
M-P 012	-1,3	-2,5	-2,5	Osteoporotic	1
O-C 013	-3	-2,8	-3	Osteoporotic	1
T-C 014	-2	-2	-2	Osteopenic	0
L-B 015	-1,8	-1,5	-1,8	Osteopenic	0
G-M 016	-1,2	-2,5	-2,5	Osteoporotic	1
N-G 017	-2,7	-1,8	-2,7	Osteoporotic	1
A-B 018	-2,6	-2	-2,6	Osteoporotic	1
G-P 019	-3,2	-1,7	-3,2	Osteoporotic	1
R-G 020	-1,2	-1,6	-1,6	Osteopenic	0
D-N 021	-3,6	-2,2	-3,6	Osteoporotic	1
A-C 022	-1,8	-1,6	-1,8	Osteopenic	0
M-M 023	-1,4	-1	-1,4	Osteopenic	0
M-S 024	-2,1	-2,1	-2,1	Osteopenic	0
S-G 025	0,6	-1,3	-1,3	Osteopenic	0
L-T 026	-2,1	-1,1	-2,1	Osteopenic	0
M-C 027	-2,4	-1,3	-2,4	Osteopenic	0
E-S 028	-2,7	-2,6	-2,7	Osteoporotic	1
M-G 029	-2,2	-1,8	-2,2	Osteopenic	0
A-C 030	0,8	-1,9	-1,9	Osteopenic	0
M-F 031		-1,3	-1,3	Osteopenic	0
M-M 032	-2,1	-2,4	-2,4	Osteopenic	0
M-P 033	-3,3	-1,4	-3,3	Osteoporotic	1

MR-B 034	-1,6	-1,7	-1,7	Osteopenic	0
G-C 035	-2,8	-2,7	-2,8	Osteoporotic	1
A-G 036	-3,6	-3,8	-3,8	Osteoporotic	1
G-S 037	-1,9	-2,1	-2,1	Osteopenic	0
R-S 038	-2,6	-1,5	-2,6	Osteoporotic	1
G-S 039	-2	-1,4	-2	Osteopenic	0
G-C 040	-3,3	-1,9	-3,3	Osteoporotic	1
MN-T 041		-1,9	-1,9	Osteopenic	0
F-D 042	-1,6	-1,8	-1,8	Osteopenic	0
M-A 043	-2,1	-3	-3	Osteoporotic	1
MP-G 044	-1,6	-2,2	-2,2	Osteopenic	0
A-D 045	-1	-1,5	-1,5	Osteopenic	0
R-C 046	-1,1	-2,1	-2,1	Osteopenic	0
L-R 047	-1,9	-1,9	-1,9	Osteopenic	0
D-B 048	-1,6	-0,9	-1,6	Osteopenic	0
C-P 049	-2,2	-2	-2,2	Osteopenic	0
A-P 050	0,1	-0,7	-0,7	Healty	0
M-B 051	-1,9	-1,4	-1,9	Osteopenic	0
M-M 052	-0,9	-1,6	-1,6	Osteopenic	0
PKS 053		-2,5	-2,5	Osteoporotic	1
AMF 054		-3,1	-3,1	Osteoporotic	1
K-D 055		-2,7	-2,7	Osteoporotic	1
C-M 056		-2,5	-2,5	Osteoporotic	1
PFF 057		-3,3	-3,3	Osteoporotic	1
G-G 058		-2,7	-2,7	Osteoporotic	1
NMF 059		-3,7	-3,7	Osteoporotic	1
DJG 060		-2,6	-2,6	Osteoporotic	1
DIG 061		-3,8	-3,8	Osteoporotic	1
LMK 062		-3,2	-3,2	Osteoporotic	1
J-W 063		-2,5	-2,5	Osteoporotic	1
J-B 064		-3,5	-3,5	Osteoporotic	1
MBM 065		-2,5	-2,5	Osteoporotic	1
V-F 066		-3,6	-3,6	Osteoporotic	1
SAR 067		-2,7	-2,7	Osteoporotic	1
JAL 068		-2,9	-2,9	Osteoporotic	1

A-S 069		-3,6	-3,6	Osteoporotic	1
A-L 070		-2,5	-2,5	Osteoporotic	1

Tab. 17 T-scores and diagnostic classification in healty, osteopenic and osteoporotic. Healthy and osteopenic patients have been classified as "0" and osteoporotic as "1".

Patients	Morphometric Indices						Diagnosis
	KLEMETTI INDEX	MCW mm	PMI mm	M/M mm	AI mm	GI mm	0=healthy/osteopenic ; 1=osteoporotic
L-Z 001	C2	4,57	0,40	3,15	3,27	1,96	1
G-P 002	C2	3,62	0,26	2,56	2,48	1,22	0
M-M 003	C2	3,14	0,22	1,70	3,26	1,52	0
A-B 004	C2	1,97	0,15	1,65	2,56	1,61	0
R-L 005	C2	2,55	0,19	1,69	2,88	1,41	0
C-G 006	C3	1,95	0,17	1,79	2,02	1,15	0
L-M 007	C2	3,73	0,34	1,88	2,58	1,58	0
T-C 008	C2	3,21	0,30	1,88	3,09	1,91	1
M-G 009	C1	3,84	0,30	2,24	2,80	1,37	0
S-M 010	C3	4,27	0,33	2,05	3,43	1,26	1
O-C 011	C3	2,33	0,29	2,48	3,15	1,35	1
M-P 012	C3	2,92	0,24	2,62	3,15	1,69	1
O-C 013	C3	0,97	0,08	2,15	2,19	1,50	1
T-C 014	C2	4,20	0,62	3,54	2,42	2,08	0
L-B 015	C2	3,83	0,33	2,64	3,49	4,49	0
G-M 016	C2	3,03	0,24	2,67	2,11	1,71	1
N-G 017	C3	2,73	0,20	2,03	2,35	1,61	1
A-B 018	C1	3,36	0,25	2,41	3,34	1,98	1
G-P 019	C3	1,93	0,15	2,01	2,06	0,97	1
R-G 020	C2	1,96	0,15	1,57	1,50	1,48	0
D-N 021	C2	2,44	2,33	2,50	2,16	1,12	1
A-C 022	C3	3,02	0,34	3,23	2,24	1,91	0
M-M 023	C1	3,73	0,27	1,85	1,57	1,51	0
M-S 024	C1	4,65	0,36	2,12	3,10	1,28	0
S-G 025	C1	3,80	0,26	2,08	2,83	1,51	0
L-T 026	C1	4,37	0,37	2,99	2,99	1,59	0
M-C 027	C2	3,14	0,22	2,02	2,53	1,80	0
E-S 028	C1	4,20	0,39	1,41	3,30	1,99	1
M-G 029	C2	4,13	0,23	1,28	1,69	1,07	0
A-C 030	C1	5,39	0,42	2,28	3,86	1,33	0

M-F 031	C1	4,55	0,32	2,09	2,55	1,69	0
M-M 032	C2	3,89	0,31	2,27	3,10	1,13	0
M-P 033	C3	4,20	0,31	2,37	3,36	1,42	1
MR-B 034	C1	4,34	0,31	1,90	3,31	1,80	0
G-C 035	C3	1,23	0,10	1,81	1,99	0,84	1
A-G 036	C2	3,32	0,26	1,86	2,39	1,41	1
G-S 037	C2	3,78	0,35	2,23	2,73	1,00	0
R-S 038	C2	3,28	0,34	2,44	3,00	1,45	1
G-S 039	C2	5,02	0,40	1,95	4,72	2,94	0
G-C 040	C3	2,33	0,17	1,95	3,42	2,01	1
MN-T 041	C3	3,53	0,25	2,35	4,11	2,10	0
F-D 042	C1	4,23	0,41	3,45	3,17	1,76	0
M-A 043	C2	2,09	0,13	1,27	2,41	1,62	1
MP-G 044	C3	3,39	0,20	1,46	1,99	0,84	0
A-D 045	C2	3,48	0,21	1,89	2,51	1,28	0
R-C 046	C2	3,12	0,20	1,99	1,53	1,14	0
L-R 047	C3	2,67	0,20	1,94	2,76	1,47	0
D-B 048	C2	4,19	0,31	2,06	2,93	2,01	0
C-P 049	C2	3,43	0,31	2,18	1,61	1,85	0
A-P 050	C3	3,31	0,21	1,21	2,60	1,75	0
M-B 051	C3	2,13	0,16	2,34	2,17	1,47	0
M-M 052	C2	2,06	0,18	2,75	3,15	75,37	0
PKS 053	C2	n/d	n/d	n/d	n/d	n/d	1
AMF 054	C3	n/d	n/d	n/d	n/d	n/d	1
K-D 055	C3	n/d	n/d	n/d	n/d	n/d	1
C-M 056	C2	n/d	n/d	n/d	n/d	n/d	1
PFF 057	C3	n/d	n/d	n/d	n/d	n/d	1
G-G 058	C3	n/d	n/d	n/d	n/d	n/d	1
NMF 059	C3	n/d	n/d	n/d	n/d	n/d	1
DJG 060	C3	n/d	n/d	n/d	n/d	n/d	1
DIG 061	C3	n/d	n/d	n/d	n/d	n/d	1
LMK 062	C3	n/d	n/d	n/d	n/d	n/d	1
J-W 063	C1	n/d	n/d	n/d	n/d	n/d	1

J-B 064	C3	n/d	n/d	n/d	n/d	n/d	1
MBM 065	C3	n/d	n/d	n/d	n/d	n/d	1
V-F 066	C3	n/d	n/d	n/d	n/d	n/d	1
SAR 067	C2	n/d	n/d	n/d	n/d	n/d	1
JAL 068	C1	n/d	n/d	n/d	n/d	n/d	1
A-S 069	C3	n/d	n/d	n/d	n/d	n/d	1
A-L 070	C2	n/d	n/d	n/d	n/d	n/d	1

Tab. 18 Panoramic morphometric indices: Klemetti index, mandibular cortical width (MCW), panoramic mandibular index (PMI), mental ratio (M/M), antegonial index (AI), gonial index (GI).

Patient	N of teeth loss	% teeth loss	% teeth with mobiilty	furcations	T-score	% sites with PPD≥5mm	% sites with PPD <5 mm	% sites with REC>3mm	% sites with REC≤3mm	% sites with AL 1-2 mm	% sites with AL 3-4 mm	% sites with AL ≥5 mm
			(28 = 100 %)	(no/grade I=0; grade II/III=1)	(0=healthy/osteopenic 1= osteoporotic)							
L-Z 001	3	10,7	8	0	1	0	100	0	100	11,3	83,4	5,3
G-P 002	7	25	62,5	0	0	13,9	86,1	0	100	15,9	54,9	29,2
M-M 003	22	78,6	0	0	0	13,9	86,1	18	82	6	36,4	57,6
A-B 004	28	100	0	0	0	0	100	0	100	16,7	83,3	0
R-L 005	28	100	0	0	0	16,7	83,3	6	94	3	41	56
C-G 006	28	100	0	0	0	33,3	66,7	0	100	36,7	30	33,3
L-M 007	4	14	4	0	0	1,3	98,7	7,7	92,3	24,3	48,1	27,6
T-C 008	26	92,8	0	0	1	0	100	83,3	16,7	0	0	100
M-G 009	16	57,1	0	0	0	5,3	94,7	1,7	98,3	19,4	40,3	40,3
S-M 010	6	21,4	56,5	1	1	5,8	94,2	14,5	85,5	3,6	25,4	71
O-C 011	14	50	0	0	1	3,6	96,4	4,7	95,3	13,1	53,6	33,3
M-P 012	3	10,7	23,1	0	1	7	93	1,9	98,1	31,4	50	18,6
O-C 013	5	17,9	26,9	0	1	3,8	96,2	0	100	26,5	57,6	15,9
T-C 014	28	100	0	0	0	0	0	0	0	0	0	0
L-B 015	3	10,7	48	1	0	27,3	72,7	1,3	98,7	8,6	44,7	46,7
G-M 016	3	10,7	46,1	1	1	12,8	87,2	0,6	99,4	20,5	47,4	32,1
N-G 017	22	78,6	0	0	1	19,4	80,6	8,3	91,7	2,8	22,2	75
A-B 018	1	3,6	0	0	1	0,6	99,4	0,6	99,4	25,6	63,5	10,9

G-P 019	10	35,7	33,3	0	1	18,7	81,3	0	100	17,7	35,4	46,9
R-G 020	21	75	0	0	0	8,3	91,7	0	100	10	78,3	11,7
D-N 021	2	7,1	51,8	1	1	9,8	90,2	6,2	93,8	19,1	46,9	34
A-C 022	6	21,4	0	0	0	6,7	93,3	0	100	29,3	63,3	7,4
M-M 023	11	39,3	4,7	0	0	21,7	78,3	0	100	22,5	54,2	23,3
M-S 024	5	17,9	17,2	0	0	5,7	94,3	1,1	98,9	25,3	53,4	21,3
S-G 025	5	17,9	0	0	0	4,2	95,8	0	100	38,2	56,9	4,9
L-T 026	3	10,7	20	1	0	15,3	84,7	0	100	4,6	50,7	44,7
M-C 027	14	50	40,9	0	0	44,7	55,3	2,3	97,7	3	28	69
E-S 028	12	42,8	0	0	1	0	100	4,2	95,8	22,9	42,7	34,4
M-G 029	8	28,6	31,8	0	0	3	97	5,3	94,7	22,7	58,3	19
A-C 030	17	60,7	50	0	0	52,6	47,4	0	100	2,6	26,9	70,5
M-F 031	0	0	0	0	0	1,6	98,4	0	100	57,5	40,9	1,6
M-M 032	6	21,4	10	0	0	0,5	99,5	0,5	99,5	57,8	37,2	5
M-P 033	1	3,6	0	0	1	0,6	99,4	0	100	46,3	51,8	1,9
MR-B 034	4	14,3	0	0	0	5,3	94,7	3,3	96,7	20	63,3	16,7
G-C 035	18	64,3	50	0	1	6,7	93,3	3,3	96,7	16,7	70	13,3
A-G 036	0	0	3,6	0	1	2,9	97,1	0	100	22,1	69,6	8,3
G-S 037	5	17,9	18,5	1	0	22,2	77,8	0,6	99,4	17,9	58,6	23,5
R-S 038	13	46,4	18,7	0	1	7,3	92,7	0	100	8,4	70,8	20,8
G-S 039	13	46,4	0	0	0	2,6	97,4	0	100	20,2	71,9	7,9
G-C 040	8	28,6	0	0	1	0	100	0	100	24,2	71,2	4,6

MN-T 041	0	0	0	0	0	2,4	97,6	0	100	35,7	60,7	3,6
F-D 042	4	14,3	34,6	0	0	16,7	83,3	0	100	12,3	50	37,7
M-A 043	26	92,8	33,3	0	1	58,3	41,7	5,5	94,5	2,8	30,5	66,7
MP-G 044	28	100	0	0	0	0	0	0	0	0	0	0
A-D 045	3	10,7	4	1	0	6,7	93,3	0	100	20	73,3	6,7
R-C 046	22	78,5	0	0	0	25	75	5	95	2	55	43
L-R 047	6	25	41,6	1	0	30,5	69,5	0	100	18	38,1	43,8
D-B 048	8	28,6	7,6	0	0	0,6	99,4	0	0	41,6	51,2	7,1
C-P 049	6	21,4	76,9	1	0	12,8	87,2	7	93	16,6	42,9	50,5
A-P 050	28	100	0	0	0	14	86	1,2	98,8	21,7	56,4	21,8
M-B 051	5	17,8	55,5	0	0	14,8	85,2	1,8	98,2	12,3	47,5	40,2
M-M 052	5	17,9	24	1	0	14,7	85,3	0,7	9,3	12	44	44
PKS 053	1	3,6	0	1	1	27,5	72,5	1,5	98,5	8	49	43
AMF 054	2	7,1	0	1	1	5,7	94,3	0	100	60,9	30,1	9
K-D 055	1	3,6	0	0	1	5,2	94,8	0	100	32,8	60,3	6,9
C-M 056	2	7,1	18,5	0	1	9,3	90,7	0	100	21	61,7	17,3
PFF 057	4	14,3	60,7	0	1	4,2	95,8	0	100	27,4	56,5	16,1
G-G 058	9	32,1	52,6	0	1	0,9	99,1	2,6	97,4	9,6	59,6	30,8
NMF 059	8	28,6	30	0	1	8,3	91,7	0	100	12,5	64,2	23,3
DJG 060	5	17,8	45,8	0	1	1,4	98,6	4,8	95,2	19,4	54,9	25,7
DIG 061	9	32,1	21	0	1	1,7	98,3	0,9	99,1	25,5	59,6	14,9
LMK 062	2	7,1	38,5	0	1	1,3	98,7	0	100	33,3	53,4	12,8

J-W 063	2	7,1	17,2	0	1	0,6	99,4	0	100	35,6	59,8	4,6
J-B 064	4	14,3	0	0	1	0	100	0	100	42	52	6
MBM 065	6	21,4	54,5	1	1	5,5	94,5	4,5	95,5	18,2	52,3	29,5
V-F 066	5	17,8	4	0	1	0	100	0	100	27,3	68,7	4
SAR 067	1	3,6	0	0	1	2,7	97,3	0	100	51,6	43	5,4
JAL 068	2	7,1	34,6	0	1	0	100	1,3	98,7	7	64,7	28,3
A-S 069	4	14,3	3,6	1	1	2,4	97,6	0,6	99,4	2,4	66,7	30,9
A-L 070	8	28,6	5	1	1	3,3	96,7	5	95	5	68,3	26,7

Tab. 19 Periodontal data collection: Number/percentage of teeth loss, percentage of teeth with augmented mobility, furcation involvement, percentage of sites with pocked probing depth (PPD), recession (REC) and clinical attachment level (AL)

6 DISCUSSION

According to the revision of the literature that it has been performed, four out of the five studies included reported a significant correlation between BMD measured at different systemic skeletal sites and mandible BMD. Cakur et al (172) reported in 2009 no evidence of any relationship between systemic and mandibular BMD. However, we need to be very cautious on drawing conclusions, since these studies are not easily and satisfactory comparable due to several confounding factors such as different techniques used to measure BMD and the different anatomic sites considered.

Since the heterogeneity of the studies and considering that the meta-analysis was performed taking into account only two-studies, it is possible to state that the significance of these data can be questionable.

For these reasons, a clinical trial has been designed in order to investigate the correlation between systemic bone mineral density (BMD) and jawbones condition, using a set of specific radiographic morphometric indices proposed in the last decades. Moreover, since the enrolment has been performed in a Dental Hospital, it was decided to investigate the relationship between BMD and periodontal status in this group of selected patients. The correlation between t-scores and periodontal indices has already been proposed in scientific literature but, similarly to the correlation between BMD and jawbone density, the evidence is poor and characterized by a large heterogeneity of the studies. The available literature on a possible correlation has been reviewed in Chapter 3.

6.1 PANORAMIC MORPHOMETRIC INDICES FOR DETECTING REDUCED BMD

Several clinical studies investigated the relationship between bone density measured in different systemic skeletal sites and in the jawbones in subjects with different T scores. Although many of these studies have found a positive correlation (132-139), others have reported that jawbone density is not, or only to a limited degree, correlated to the density in other systemic sites (140-142). It has also been demonstrated that that there are no

differences in jawbone density between normal and osteopenic/osteoporotic subjects (143, 144).

The available literature on the accuracy of panoramic morphometric indices in detecting reduced BMD has been systematically reviewed in Chapter 3. In the past 20 years several evidences have been published on the accuracy of qualitative/quantitative panoramic indices in screening for reduced skeletal density (either osteopenia or osteoporosis) (173). Considering the high percentage of people attending regular dental visits and the fact that panoramic radiographs of the jawbones are nowadays a common procedure during routine dental check-ups or before several dental treatments (174-176), it would be of great clinical value if dentists could opportunistically use panoramic X-rays to identify patients at high risk of osteoporosis and at least refer them to metabolic bone diseases clinic.

In the study presented, the Klemetti index (KI), the qualitative index, has been evaluated on the whole sample of 70 patients, while panoramic morphometric quantitative indices such as mandibular cortical width (MCW), panoramic mandibular index (PMI), mandibular ratio (M/M), antegonial index (AI) and gonial index (GI) have been measured on 51 patients due to technical radiological issues.

In line with several researchers in the field, we agree that the basal area of the mandible posterior to the mental foramen is probably the only part of the jaws with reasonably suitable characteristics to be a standard site for BMD measurements, since it has small inter- and intra-individual variations in anatomical size, shape, bone structure and function (177), although it has to be remembered that the mandibular foramen cannot be detectable in all conditions.

Mandibular cortical width represents the thickness of the mandibular cortex and is usually measured in the mental foramen region. Regardless the limitations of this study due to the sample size, the statistical analysis has shown a significant correlation between MCW and a status of osteoporosis and a cut-off value has been set at 3,3 mm with a sensitivity and specificity of 72,2 % and 64,7 %, respectively. According to the literature,

MCW has showed a better specificity rather than sensitivity in detecting people with reduced BMD, since 90% of people with MCW ≥ 4 mm could be correctly identified as having a normal BMD (157).

Regarding the qualitative measurement, Klemetti index has been evaluated by two different blinded examiners and correlated to the status of bone health or osteoporosis of the patient. KI qualitatively classifies the mandibular cortex distally to the mental foramen in 3 categories according to the presence of erosions. The presence of cortical erosion (C3 vs. C1-C2) has produced a sensitivity of 61,1 % and a specificity of 79,4 % with a positive predictive value of 75,9 % and a negative predictive value of 65,9 %. In the revision of the literature, the presence of any kind of cortical erosion (C2+C3 categories, as assessed with KI) has seemed a sensitive tool to detect reduced BMD, since it was associated with at least osteopenia in approximately 80% of the cases (160).

Examining the clinical trial results of MCW and KI, a possible correlation between the two main indices has been tested and a strong correlation has been found (p value = 0,001). According to this finding, a correlation between the two cut-off values that identify a healthy patient (MCW>3,3mm and KI=C1/C2) and t-scores has been investigated. The correlation has shown an increase in sensibility and specificity till the values of 88,8 % and 58,8 %, respectively, but, most important, demostrated a negative predictive value of 91 %. These indices can be considered potentially useful to screen for reduced skeletal BMD if used in combination and in particular to identify a healthy patient in more than 90 % of cases.

Even if scientific literature has demonstrated that panoramic mandibular index (PMI) seem the most accurate and consistent quantitative tool to screen for reduced BMD (sensitivity and specificity >70%) (161), this work PMI has reached no significance if related to the t-scores values to screen for healthy or osteoporotic patients. Most of the studies reported a cutoff value of 0,3, with levels of sensitivity and specificity in detecting individuals with reduced bone density (T score <–1) ranging from 40.8% to 100% and from 47% to 88%, respectively.

In the same way, antegonial index, gonial index and mandibular ratio have shown an insufficient significance as predictor tools. Literature data on these "secondary" indices is poor, because all the available meta-analysis have been performed on MCW, PMI and KI. Moreover, several studies reporting a positive correlation between skeletal and jawbone BMD have not distinguished between osteoporotic and healthy patients when reporting the correlation coefficients, thus further limiting the possibility of drawing robust conclusions.

Another limitation we found during the literature review was related to the fact that all studies have considered only mandibular data, without taking into account the maxilla. It is well known that in osteoporotic subjects bone loss is not uniform and that the trabecular bone is earlier and more deeply affected than the cortical bone (29, 178). The mandible has a better resemblance with the femur neck (179, 180), where fractures are primarily caused by a loss in cortical rather than trabecular bone (181, 182). Considering that the maxilla is mainly made of trabecular bone, it is likely that bone density measured at this site would have been better related to vertebral osteoporosis. However, the lack of stable referral points (like the mental foramen in the mandible) makes it challenging to evaluate standardized sites in the maxilla.

When dealing with jawbones it should also be kept in mind that they display unique anatomic characteristics in comparison with other bones of the skeleton, owing for example to their special relationship with teeth and the distinction between the more stable basal bone and the alveolar bone, which atrophies after teeth are lost (183, 184). It may be hypothesized that these and other anatomical/physiological peculiarities of the jaws can somehow account for differences in bone metabolism response (185).

6.2 PERIODONTAL EXAMINATION DATA AND BMD

Periodontitis is an infection-induced inflammation of the structures around the tooth resulting in loss of its soft tissue attachment and surrounding bone mass, finally resulting in tooth loss. A relation between osteoporosis and periodontitis has also been postulated in literature since many years. Osteoporosis being a systemic disease, leads to loss of

bone stock not only from spine and appendicular skeleton but also from the alveolar bone. Thus, osteoporosis is expected to hasten the process of bone loss in chronic periodontitis (186).

Osteoporosis is well known in postmenopausal women with prevalence as high as 50% (187) and the postmenopausal status is also associated with increased severity of periodontitis with prevalence as high as 30% (102, 188). Periodontitis is clinically measured in terms of CAL, PPD, and alveolar bone loss. Other periodontal indices that researchers have to take into account are recession, tooth mobility, furcations involvement and tooth loss.

In the present study, all the aforementioned parameters have been recorded from 70 patients in order to analyze the prevalence of periodontal disease in a population of post-menopausal women. The correlation between periodontal status and BMD has been statistically investigated comparing periodontal indices and the worst t-score value.

Pocket probing depth has been recorded with a 6-point sounding and a group of 51 patients has been analyzed. A statistical significant correlation has been demonstrated between periodontal probing depth > 5 mm and BMD t-scores. It is of primary importance to underline that the direction of the correlation has been towards the healthy group. These data show that osteoporotic patients exhibit a better periodontal condition in terms of periodontal sounding in respect of the patients with a t-score > -2,5.

Similarly, clinical attachment loss, considered as PPD added to REC value, has been correlated to the diagnosis of osteoporosis. Sites have been classified in three clusters according the progressive clinical attachment loss. The group with CAL of 1-2 mm has been considered as periodontal health and the correlation has shown no significance, similarly, the group with CAL > 5 mm, representative of the population with a severe periodontal destruction has shown a poor correlation.

Interestingly, the group with CAL of 3-4 mm, representative of the larger part of the population has shown a statistically significance if correlated to BMD. Accordingly to the PPD value, the correlation is towards the patients diagnosed as healthy.

These two parameters are considered, in the scientific literature, two of the most predictable tools to identify a correlation between osteoporosis and periodontitis (189).

Several studies have demonstrated that postmenopausal women, with lower bone mineral density, have a higher number of Decayed-Missing-Filled-Teeth and, as the age of the postmenopausal subject increases, the number of teeth decreases and the Decayed-Missing-Filled-Teeth score increases (189). Old osteoporotic women most likely have periodontal disease compared to those without osteoporosis. An increasing in age and a decreasing in female sexual hormones are related to an increasing in bone loss. Oral signs, Body Mass Index and age can be used as criteria indicating osteoporosis' risk. A recent systematic review by Goyal et al. has favored a strong association between the central BMD and CAL.

Findings from this research are in disagreement with data of the literature, and PPD ≥ 5 and CAL 3-4 mm have been demonstrated associated to a condition of healthy systemic bone density, and consequently, osteoporotic patients have shown better periodontal conditions (190). These results could seem controversial but it is crucial to analyze two key-factors: the recruiting pattern and the sample size. All the patients have been enrolled in a Dental hospital, and it is plausible that they have been used to attend the dental hospital to receive dental treatments. In addition, it is plausible that patients that have received a diagnose of osteoporosis and, in several cases, a treatment plan that provides the assumption of medications such as bisphosphonates, have a particular attention and care to their health. The oral health of this type of patients is periodically controlled and the periodontal status is usually maintained with a proper periodontal recall scheme.

The second aspect to take into account is the power of the study. The sample size of the research has been set to obtain a statistical power according to the findings from the review of Calciolari et al. and it has been based on the primary outcomes: to assess the feasibility of using dental panoramic indices to screen for post-menopausal osteoporosis and to assess the sensitivity and specificity of quantitative and qualitative panoramic indices (165). For this reason, the study is underpowered in assessing if periodontal indices are correlated to BMD. Moreover, at the moment of the data analysis, the recruitment has been still ongoing and only data from 70 patients were available. According to these considerations, all the results from the periodontal data examination

have to be confirmed with further studies with a larger sample of patients and with a strict recruitment outline.

Regarding the others periodontal indices, only the number and the percentage of tooth loss have demonstrated a correlation that has been confirmed statistically. In this case the tendency of missing teeth has been towards the osteoporotic groups. These data are in contrast to the aforementioned periodontal findings, and this strengthen the hypothesis that the sample is too small to draw meaningful conclusions.

6.3 SAMPLE SIZE AND RECRUITMENT PATTERN

As previously described, the recruitment of the patient has been done within a pool of post-menopausal women attending the Centre of Dentistry at Parma University. According to the findings here shown, it is plausible that a recruitment-centre related bias could be present and of some importance. A large portion of the patients, that regularly attends the dental hospital, follows a personalized recall program in order to control the periodontal status over time. In particular, periodontal compromised patients undergo a supportive periodontal therapy according to the periodontal risk assessment tool.

Furthermore, the study has been designed to reach a statistical power of 80 % in identifying morphometric panoramic indices as tools to diagnose osteoporosis, according to the data from the review by Calciolari et. al (2015), and the sample number has been set at 124 (165). As evidenced by the critical review of the literature, the proper sample size to investigate the prevalence of periodontal disease in osteoporotic patients has to be wider. Moreover, since the study is still going, only 70 patients have been included in the study, making the sample size even less powered. For these reasons, further studies with a proper sample size calculation and without population selection biases are needed and are already ongoing, in order to obtain significative results.

6.4 CONFUNDING FACTORS

Some confounding factors could be considered to better understand the results and to draw sensible conclusions.

6.4.1 Age

The inclusion criteria of the study have set at 65 years old the minimum age to be eligible for the study. This has been decided according to Italian national health system guidelines that considers above 65 years, the cut-off age from which consider the risk of osteoporosis as high. It is important to know that 50 years old post-menopausal women could be easily find within a normal population and, with these inclusion criteria, a cluster of patient from 50 to 65 years old are not involved. Furthermore, older patients show different interfering medical conditions such as, bone metabolism, medications, concomitant pathologies, dental status, tooth loss, etc. and this aspect could be crucial in a statistical analysis. In fact, according to the literature a 30% prevalence of osteoporosis amongst post-menopausal women attending a dental hospital, has been speculated. Anyhow, considering the age of the eligible patients, the prevalence of osteoporosis in the population could be higher than the 30%. This consideration has been confirmed by our data that have shown a prevalence of osteoporotic patients within the sample of 50 %.
At the end of the study, a Fisher test could be necessary in order to exclude the age as a confounding variable.

6.4.2 Smoke

The available literature about panoramic indices does not take into account the condition of smoker/non-smoker of the patients. However, smoke is considered as a risk factor for the progression of the periodontitis and has been demonstrated strictly correlated to periodontal conditions. For this reason, smoke could be considered as a confounding variable in the analysis of the correlation between periodontal status and BMD but the

Fisher test has shown no correlation. Nevertheless, the small sample size suggests that a second test executed on a larger population could be more effective.

6.4.3 Medications

During the past decade, increasing concern has developed towards a severe, although rare, complication of anti-osteoporotic drugs named osteonecrosis of the jaw (ONJ).
It is usually associated to bisphosphonates, in particular nitrogenous-based and intravenous one and the first documented ONJs date back to 2003 and since then an increasing number of publications has focused on this problem. Since then, other drugs, all able to inhibit bone resorption, such as denosumab or Thor's inhibitors have been considered involved in the pathogenesis of ONJs (191). Recently pubblished papers have demonstrated a correlation between BP's and better periodontal conditions .
The exact mechanism leading to ONJ is still unknown, as well as the reason why this condition develops only in the maxilla-mandibular complex. Micro damage accumulation, infection and soft tissue toxicity (at least for BPs) have all been suggested to play a role in the development of ONJs (192).It is also important to think that the oral cavity presents with unique characteristics compared to other bones of the body, as the presence of teeth allows a direct connection between the bone and the exterior. Moreover, dental and periodontal infections and dento-alveolar trauma may be able to trigger this condition (193). It is important to notice that recently pubblished papers have suggested a possible correlation between BFs and better periodontal conditions (194). For all these reasons, a possible role of BPs in influencing the correlation between body mass and jawbones could be more investigated but, to statistically demonstrate any connection, the sample need to be increased.

7 CONCLUSIONS

According to our findings, although it is not indicated to prescribe an OPG with the primary aim to screen for osteopenia/osteoporosis, whenever a pantomograph is available, MCW, and KI can be a helpful and easily usable tool for the dentist to intercept patients at risk of reduced BMD, since this disease can severely interfere with any dental treatment. Moreover, considering the high negative predictive value of the combination of two indices, the dentist can easily exclude healthy patients and guide the patient at risk in a specialistic diagnostic pathway.

The possibility to use dental panoramic radiographs to identify previously undiagnosed osteoporotic patients is suggestive and seems to be supported by the systematic review presented in Chapter 3, although the average quality of the available studies is not high. Future studies should therefore account for confounding variables (e.g. medications, concomitant diseases affecting bone metabolism, demographics), ensure that examiners are blind to the skeletal BMD of the patients, and accurately evaluate the kappa of agreement for intra- and inter-examiner reliability. The optimal cut-off has been investigated in this study, by applying ROC curves and taking into consideration not only the sensitivity/specificity of the index, but also the positive and negative predictive values, which vary with the prevalence of the disease and may have a more meaningful clinical impact. Furthermore, the possibility to combine panoramic indices with well-known risk factors for osteoporosis should be addressed by future studies in order to increase their accuracy and the strength of these suggestions. Finally, the possibility to measure indices in the upper jaw needs to be investigated, although the lack of stable anatomic reference points in the maxilla might be difficult to overcome.

The willingness of general dentists to take part in training sessions and the willingness of patients to be referred to their GP or to a specialist following a dental appointment, as well as a referral pathway from general dentists directly to GPs or osteoporotic specialists should be also evaluated by future clinical projects. In the long run, a prospective study correlating panoramic indices to the risk of developing fractures, which are the real burden of osteoporosis, would add additional strength to the use of these tools, which

might potentially be added to existing fracture risk assessment tools, such as FRAX or the Italian version DeFRA.

Regarding the correlation between periodontal status and BMD it is clear that the present study is at the moment still unpowered. Further ongoing studies setting a proper statistical power and taking into account the confounding variables such as smoke, age and enrolment facility are needed. In particular, PPD and CAL need to be more investigated considering the difference between literature data and the findings from this study. Moreover, number and percentage of tooth loss are periodontal data that could be related to the t-score value. In conclusion, all these periodontal indices could give meaningful information but the enrolment bias could be avoided and the recruitment has to be more representative of the real population.

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