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# Cardiovascular Implications of Endodontic Bone Disease

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## ***Introduction***

The results we present in this thesis are the compendium of over 3 years of study and research on the interactions between dental infections, chronic inflammation and cardiovascular dysfunction.
The initial motivation of the study was a review of a theme already studied in the past, but on the basis of the current pathophysiological interpretation of atherosclerotic disease. It is well known, in fact, that the traditional degenerative genesis of this disease has been extensively revised, with the growing responsibility attributed to the inflammatory mechanism.

Atherosclerosis is a disease characterized by chronically high inflammatory state. Arterial inflammation and endothelial dysfunction play key roles at all stages of the atherothrombotic process. Inflammatory mediators are intimately implicated with the cascade of events leading to atherosclerotic plaque initiation, progression and rupture. Vascular endothelial cells express a variety of adhesion molecules that recruit monocytes when chronically exposed to noxious stimuli or pathological conditions. These monocytes apparently enter the intima under the influence of chemotactic stimuli and engulf modified LDL and cholesterol crystals (Duewell 2010). The material internalized by phagocytes induces phagolysosomal damage and subsequent leakage of contents into cytosol to activate inflammasomes and caspase 1, and consequently the generation of cytokines.
Cytokines are key mediators in the chronic vascular inflammatory response in cardiovascular disease and have been demonstrated in animal models and in humans to be potent modulators of pro-inflammatory processes. The fact that these cytokines and their receptors are highly expressed and are functional in almost all cell types implicated in the pathogenesis of atherosclerosis including smooth muscle cells, certain subset of macrophages and T cells as well as endothelium support the role of interleukins in vascular disease.
Atherosclerotic vascular disease is the primary cause of morbidity and mortality in individuals with and without type 2 diabetes mellitus (T2DM). The

progression of atherosclerosis from endothelial dysfunction to vascular occlusion or to plaque rupture is the underlying mechanism responsible for many debilitating and life-threatening diseases such as myocardial infarction, stroke and peripheral vascular disease. These diseases occur at higher frequency in T2DM patients and continue to increase despite use of current optimal therapies. T2DM is also a disease that is characterized by high inflammatory state. Pre-clinical data suggests that cytokines are of key importance in the progressive functional impairment and destruction of β-cells in type 2 diabetes.

In the first phase of the PhD project we analyzed the content of the existing scientific literature on the subject. We therefore made the review *Dental Pathology and Systemic Diseases: the Historical Background of the Focal Infection Theory*, reproduced below.

In a next step, we hypothesized the presence of a modest early vascular dysfunction in patients with AP, free from other risks and clinically manifest cardiovascular disease. For this study, we selected only the male patients younger than 40 years. These inclusion criteria were adopted to avoid the effect of aging on the evolution of vascular pathology, but also the protective effect of ovarian sex hormones on the circulatory system in women of childbearing age.

In the last phase of the current study, we studied a population of women of comparable age to that of male subjects examined previously. Patient recruitment is ongoing. For the future, we plan to involve AP patients over 55 years, of both sexes, in order to recognize a possible independent role of AP in determining cardiovascular events, regardless of the effect exerted by aging.

# ***Dental Pathologies: Apical Periodontitis and Periodontal Disease: Definitions and Differences***

The chapter of *oral pathology* includes all the affections of the oral cavity while the dental pathologies can be divided into two main threads: a) caries – pulpitis and Apical Periodontitis (its extreme consequences) and b) periodontal pathology.

Frequently, the pathologies derived from caries (caries, pulpitis and apical periodontitis) and those derived from periodontal disease (gingivitis, periodontal disease) may be considered synonymous; yet they concern two different portions of the tooth. Caries is the consequence of bacterial colonization and consequent destruction of the dental hard tissues (particularly enamel and crown's dentin) and is followed by the infection of the internal organ of the tooth (dental pulp) and, by the late, involvement of periapical tissue. The second is an infection that strikes the periodontal tissues, understood as apparatus of support of the tooth (radicular cement, parodontal ligament, alveolar bone).

It is commonly used to define Periodontal Disease as an inflammatory disease of the tissues of the teeth caused by singles or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone. A clinical consequence of this pathology is the pocket formation, the recession, or both. (Carranza's: Clinical Periodontology 2006).

This pathology is always associated to the presence of bacterial plate but its evolution is related to the immune response of the guest. The main studies concerning the pathogenesis of periodontal disease show how the chronic periodontitis is associated with specific bacterial agents. Indeed, the bacterial species found in the parodontal sites belong in high percentages to anaerobic (90%) and gram negative (75%). More specifically, the prevailing bacteria discovered at high levels in chronic periodontitis are *P. gingivalis*, *T. forsythia*, *P. intermedia*, *C. rectus*, *E. corrodens*, *F. nucleatum*, *A. actinomycetemcomitans*, *P. micros*, and *Treponema* and *Eubacterium* species.

In conclusion, recent studies attest an association between chronic periodontitis and viral microorganism of the herpes virus group, most notably Epstein- Barr virus-1 (EBV-1) and human cytomegalovirus (HCMV).

## ***Periodontal Disease: Clinical Manifestations and Diagnosis***

The clinical feature that is typical of Periodontal Disease (Periodontitis) is the presence of clinically detectable attachment loss. Moreover, this is characterized by a periodontal pocket formation and changes in the density and height of subjacent alveolar bone. However, those are not the only consequences. Indeed, in some cases recession of the marginal gingiva could come with an attachment loss, in this way concealing the disease in progress if pocket depth measurements are taken without quantifying the clinical attachment levels.

Symptoms of inflammation, such as alterations of colour, contour, consistency and bleeding on probing, are not always necessarily positive indicators of an evolving attachment loss. However, the presence of continued bleeding on probing during regular visits has proved to be a reliable sign of the occurrence of the inflammation, and potentially a subsequent attachment loss at the bleeding site. It has been noticed how the attachment loss associated with periodontitis evolves either progressively or in intermittent manifestations of disease activity. The chronic periodontitis is the most common form of this pathology, even if it exists in other two forms as well: aggressive and as a manifestation of systemic disease.

Chronic periodontitis is measured using several indexes. The first one, the Periodontal Index (PI), uses a system that measures the supporting tissues for each tooth according to a progressive scale that gives a low score to gingival inflammation and relatively high score to advanced periodontal disease. In particular, the score is from 0 to 8 where 0 is Negative 1 mild gingivitis 2 gingivitis 6 gingivitis with pocket formation and finally 8 is

considered advanced destruction with loss of masticatory. Another index is the Periodontal Disease Index (PDI). One of its main aspects concerns the examination of six preselected teeth: the maxillary right first molar, maxillary left central incisor, maxillary left first premolar, mandibular left first molar, mandibular right central incisor, and mandibular right first premolar. Then, another distinctive aspect of the PDI is the use of the *cementoenamel junction* (CEJ) as a fixed landmark for measuring periodontal attachment loss. Although the PDI is rarely used today, it still is significant in these two aspects. Extent and Severity Index (ESI) acts as a further assessment criterion. It was devised to supply relevant data regarding the proliferation and seriousness of periodontal disease among individuals and population. In conclusion, the use of radiography to determine the extent of bone loss is a pivotal aspect of the clinical diagnosis of periodontal disease. In this respect, research has demonstrated that when bone loss is measured from bite-wing radiographs as the distance from the CEJ to the alveolar crest, it is possible to define bone loss in terms of that distance in millimeters or in terms of percentage of root lenght.

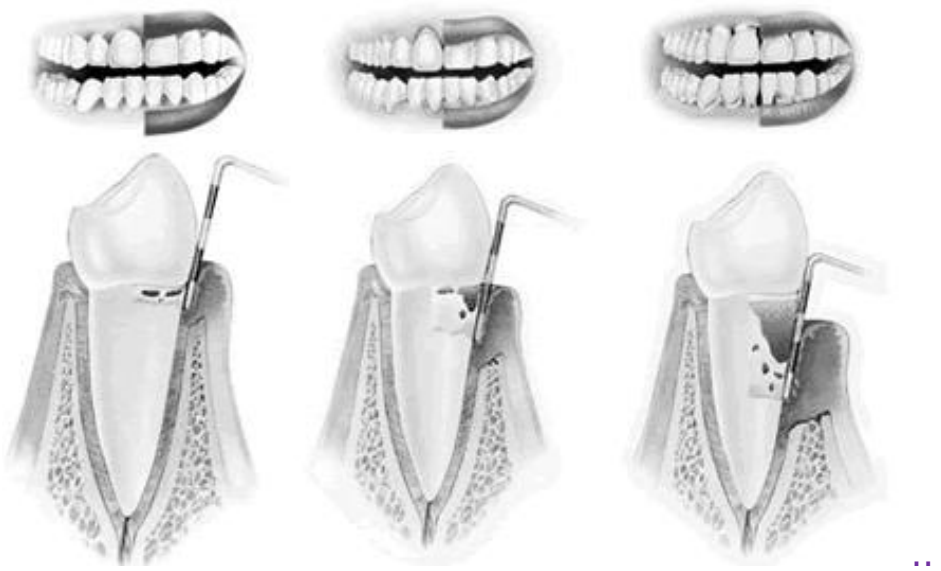

**fig. 1 Evolution of periodontal disease**

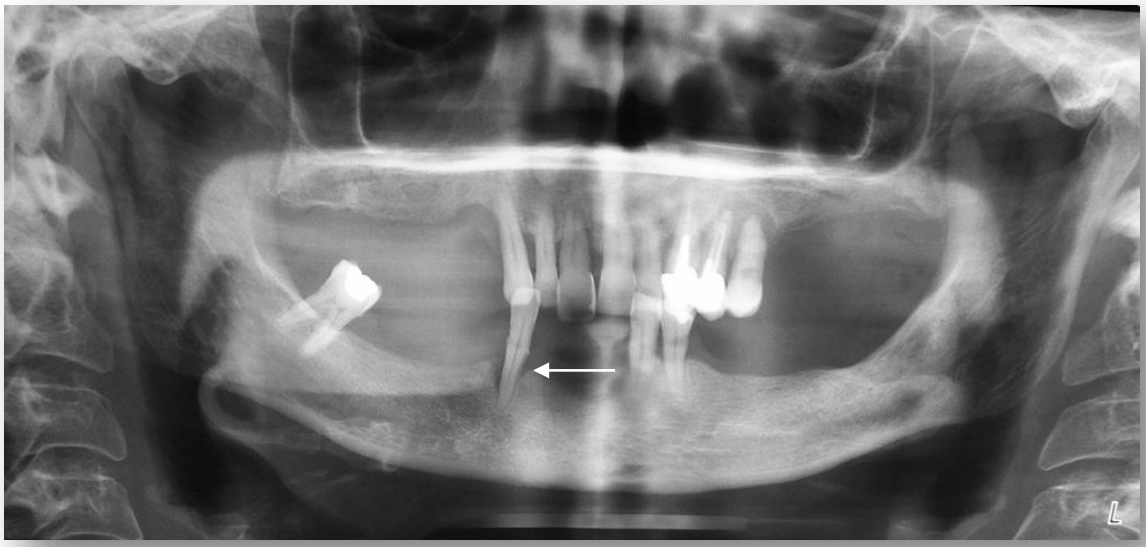

**Fig.2 Panoramic radiograph of a patient presenting with Periodontal Disease. The image shows the absence of many teeth, a generalized resorption of the alveolar bone, and loss of periodontal attachment and presence of pockets (arrow) in the residual teeth.**

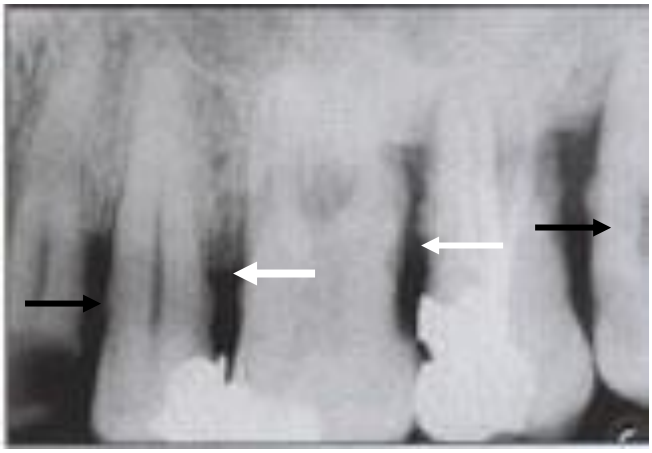

**Fig.3 Intraoral periapical radiograph showing diffused bone loss (white arrows) and presence of calculus (black arrows) involving teeth #25, 26, 27 affected by Periodontal Disease.**

Caries is perhaps the first pathology of the oral cavity; it prevailingly affects the hard tissues that compose the crown of the tooth (enamel, dentin and cement). Carious pathology consists in the progressive dissolution of the

mineral component of dentin enamel and sometimes also of the cement. It is a multifactorial pathology whose genesis is bacterial. The streptococcus mutans is the principal bacterium responsible of the caries but there are some concomitant factors tied up to the immunitary defenses of the guest, to the diet, to the very anatomy of the tooth. If not arrested, the caries notches the pulp-dentinal organ with involvement of the nervous-vascular bundle and develops in a pulpal pathology (pulpitis). Pulpitis can be reversible or irreversibile, according to the progression of the illness and the typology of symptoms. The evolution of the pulpal pathology is the periapical bony pathology or *Apical Periodontitis*.

Apical periodontitis is the result of an inflammatory response in the periapical area of teeth with necrotic pulps which act as a reservoir of infection. The role of apical periodontis is the attempt to prevent the spread of infection from the tooth to the periapical tissues and more generally to maxillary bones. It is considered the host response to bacteria and bacterial by-products in the infected root canal system. The key role in this process is played by the inflammatory reaction and the immune response. Polymorphonuclea r leukocytes (PMNs) that eventually phagocytize and kill the bacteria are the first line of response.

## ***Apical Periodontitis: Inflamatory Process in Pulp Pathology***

The pathological ways which allow the development and the progress of pulpal and periapical pathologies are non-specific inflammatory lesions.(Walton RE, Torabinejad M 2009). After lesions to the pulp tissue a cellular damage occurs, as well as the liberation of non-specific mediators of the inflammation, such as histamine, bradykinin and metabolites of arachidonic acid. Following the cellular damage, the A2 phospholipase causes the liberation of arachidonic acid from the cell membrane. The metabolization of this acid triggers the formation of various prostaglandins, such as thromboxanes and leukotrienes. Apart from non-specific inflammatory reactions, the pulpar lesion can originate

from and continued by immunological response. The potential antigenes of the inflamed gums are made of bacteria and their products; these can give to immunological reactions.

## ***Apical Periodontitis: Inflamatory Process in Periapical Pathology***

With the development and progress of periapical lesions, as well as non-specific mediators of inflammatory reactions, also an immunological reaction can take place. Pathological alterations of the pulp tissue determine the accumulation of potential antigenes within the root canal system. The presence of various immunological factors, such as IgE immune-globuline antigenes and mast cells in the pathological pulps and in the periapical tissues, indicate a type I immunological reaction. Furthermore, in human periapical lesions researchers have found different classes of immune-globuline and different classes of immunocompetent cells, such as polymorphonuclear leucocytes, macrophages, B and T cells, fragments of the C3 complement and immunocomplexes. The presence of these components in periapical lesions means that also type II and III immunological reactions have a role in the origin of these pathologies.

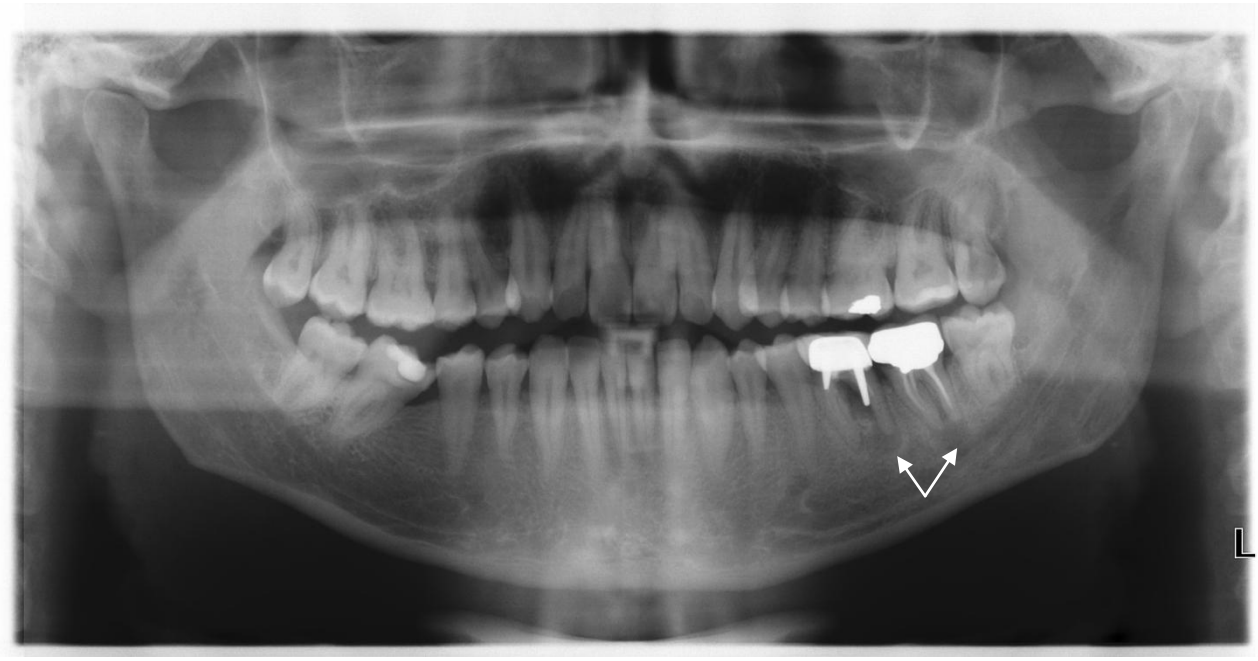

**Fig.4 Panoramic radiograph of a patient presenting with Apical Periodontitis in teeth #36 and # 37(arrows).**

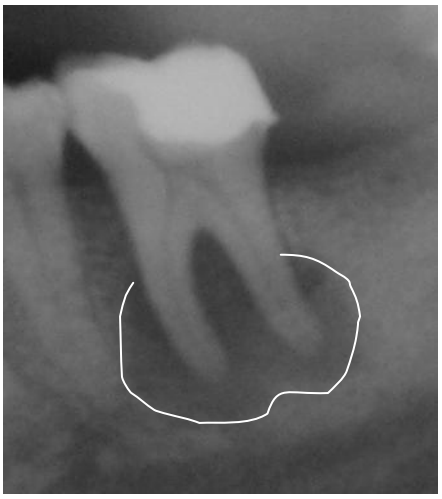

**Fig.5 An example of periapical bone lesion of endodontic origin (Apical Periodontitis) on tooth # 36 (circled). The extensive restoration present in the crown of the tooth is suggestive of the presence of a deep caries which must have caused the loss of pulp vitality and the consequent infection in the bone.**

# Chapter 1
# DENTAL PATHOLOGY AND SYSTEMIC DISEASES: THE HISTORICAL BACKGROUND OF THE FOCAL INFECTION THEORY

## ***The Focal Infection Theory***

As a rule, all pathologies affecting dental pulp and periapical tissues depend on microbial infection.(Baumgartner JC *et al.* in Ingle's Endodontics, 2008) Therefore, to effectively attend endodontic infections, it is necessary to identify the cause and effect of microbial invasion of the dental pulp and of the periapical tissues. Subsequently to a bacterial invasion of pulp tissues both non-specific inflammatory and specific immunologic response of the patient profoundly engrave the progress of the disease.

The theory of the so called "*Focal Infection"* was described in 1909, by Rosenow and was described as " *a localized or generalized infection caused by bacteria traveling through the bloodstream from a distant focus of infection"* He also introduced the concepts of *"elective localization*" as the affinity bacteria would present towards specific organs in the body. According to the literature, the first claim of the treatment of a disease associated with dental focal infection was actually by Hippocrates, who cured a case of arthritis by tooth extraction". (Baumgartner JC *et al.* in Ingle's Endodontics, 2008 p. 221). The "focal infection" had also been quoted by Miller in 1890, when he associated the presence of bacteria with pulpal and periapical disease and recommended to perform treatment of the infected root canals. In the following years Billings had reported the success of treatments of multiple affections of the body by tonsillectomies and dental extractions. The outcome of the focal infection theory as formulated by Rosenhow had several medical and dental implications in the first three decades of the 1900s: bringing the physicians into pursuing the extraction of teeth and the removal of tonsils and adenoids as the major remedy for many diseases that they assumed were caused by microbes from a distant focal infection. They

assumed that a focus of infection can interest any part of the body: in those years foci of infection were further associated with sinuses, prostate, appendix, gallbladder, and kidneys (Pallash Th.J., Wahl M.J., 2003).

With this respect, in 1910 William Hunter, a British physician, condemned the practice of dentistry in the United States stating that the practice of restorative dentistry would not treat the dental infection which should be directly eradicated by teeth extraction, since they were the cause of the many diseases of the American population (Hunter W, 1918) Hunter stated that the dental restorations were *"a veritable mausoleum of gold over a mass of sepsis."* The consequences of this trend brought to numerous extractions of teeth that could be otherwise restored.

As reported by Ingle, Weston Price published a series of experiments *in vivo* on rabbits and a series of case reports which supported the improvement obtained after dental extraction of non-vital teeth (Price WA, 1925). At the same time, the dental literature reported numerous cases in which tooth extractions were the best cure of illnesses. Although empirical, these reports wrongfully supported the continued extraction of teeth. Thus in the 1920s treatment of compromised teeth was virtually eliminated from dental education and dental practice.

## ***The Fall of Focal Infection Theory***

In the 1930s the theory of focal infection began to be undermined and by the 1940s it was abandoned leaving space to the concepts of modern dentistry; a theory according to which compromised teeth should be adequately treated and preserved. In a published paper on 200 cases of rheumatoid arthritis, Cecil and Angevine showed that no benefits were obtained on those clinical cases where tonsillectomies or dental extractions had been the elective treatments (Cecil RL, Angevine DM 1938). Afterwards, Reimann raised several critical issues: he stated that the focal infection theory with its etiologic factors could not be proved and that there was no clinical evidence

that tonsillectomies and dental extractions were actually related to better medical conditions of the patients since large groups of people with tonsils were in not worse conditions than those that had their tonsils removed. They also implied that the beneficial effects of the surgery would be easily outweighed by its harmful effects. Furthermore the foci of infection can heal after proper therapeutic measures.

### ***The Zones of Fish***

In 1939, Fish investigated the lesion of endodontic origin in the jaws (periapical lesion)(Fish E.W 1939). He described four zones of reaction formed as a consequence of bacteria implanted in the jaws of guinea pigs from the inner portion to the outer portion of the lesion: 1. Zone of Infection = inner area of the lesion where bacteria are confined by the action of polymorphonuclear leukocytes. 2. Zone of Contamination = area outside the zone of infection, characterized by the presence of inflammatory cells, but without bacteria. 3. Zone of Irritation = area of bone resorption which contains histocytes and osteoclasts. 4. Zone of Stimulation = outer layer conaining mostly fibroblasts, capillary buds, and osteoblasts. He hypothesized that if the core of the infection would be removed, that would lead to resolution of the lesion. The studies of Fish were then considered the basis for modern endodontic therapy. And the theory of focal infection was abandoned.

## Chapter 2
## Can a Dental Infection Cause a Damage To the Cardio Vascular System?

Atherosclerosis is a multifactorial disease which represents the most common cause of coronary heart disease (CHD). A strong genetic component is implicated in the genesis of atherosclerosis in association with several anatomical, physiological, and behavioral risk factors, including changes in serum lipid profile, smoking, arterial hypertension, diabetes, obesity, sedentary lifestyle, age, and gender.

Many cell types, including platelets, endothelial cells, activated monocytes, macrophages, and smooth muscle cells, are involved in the formation of atherosclerotic plaques (Hegele RA., 1996). CHD acute events are usually precipitated by thrombosis occurring at the site of atherosclerotic lesion disruption. Some CV risk markers, such as low-grade chronic inflammation, play a role in the pathogenesis of the disease (Stöllberger C, Finsterer J. 2002; Naoum JJ *et al.* 2006). Recently, bacterial and viral organisms involved in chronic inflammatory processes have also been examined (Abbas M *et al.* 2006).

The hypothesis that infection and inflammation may actively be involved in atherogenesis is supported by an increasing number of reports (Epstein SE *et al* 1999). In recent years, two patterns of association have emerged: 1. a connection between a chronic low-grade inflammation and/or infection and the slow progression of atherosclerosis and 2. a relationship between an acute systemic inflammatory response and a temporarily increased risk of severe CV events. A renewed interest in the role of infections is currently being developed, with regard to various infectious pathogens, such as Helicobacter pylori and Chlamydia pneumoniae (Patel P *et al.* 1995; Martin-de-Argila C *et al* 1995; Cook PJ *et al.* 1998). Dental infections may represent a favorable background for atherosclerosis and CVD (Beck JD *et al.* 1999; Niedzielska I *et al.* 2008). Two are the landmark dental infectious diseases: periodontal disease and apical periodontitis. Periodontal diseases are those diseases that affect one

or more of the periodontal tissues supporting the tooth (alveolar bone, periodontal ligament, cementum, gingiva) (Armitage GC 1999). Periodontal disease is divided into two main categories: 1. Periodontitis: an inflammatory disease of the supporting tissue of the teeth caused by groups of specific microorganisms, resulting in the progressive destruction of the periodontal ligament and the alveolar bone, with pocket formation, recession, or both; 2. Gingivitis: a dental plaque-induced gingival disease and non-plaque induced gingival disease. Periodontal infection is initiated by specific invasive oral pathogens (aerobic and anaerobic bacteria) that colonize dental plaque biofilms on the root surface of the tooth. Biofilms, in general, have an organized structure, composed of bacteria in a matrix of salivary glycoproteins and extracellular polysaccharides. Local and systemic factors can also modulate an individual's susceptibility to apical periodontitis.

In general, clinical signs of gingivitis are characterized by the presence of any of the following: redness and sponginess of the gingival tissue, bleeding on provocation, changes on contour and presence of calculus or plaque with no radiographic evidence of crestal bone loss. Clinical signs that suggest the presence of periodontal pockets include a bluish red, thickened marginal gingiva; a bluish red, vertical zone from the gingival margin to the alveolar mucosa; gingival bleeding and suppuration; tooth mobility. The symptoms include localized pain or pain “deep in the bone”. The only reliable method of locating periodontal pockets and determining their extent is careful probing of the gingival margin along each tooth surface. The clinical feature that distinguishes periodontitis from gingivitis is the presence of clinically detectable tooth attachment loss (Fig. 1). Treatment of periodontal diseases are based on: extraction of hopeless teeth; oral hygiene, removal of calculus and root planning; surgical therapy and maintenance (Carranza's: Clinical Periodontology 2006). Apical periodontitis is caused by bacteria (in association with viruses and fungi) residing inside the root canal/s (endodontium) of the diseased teeth, and organized in a biofilm, as a consequence of pulpal infection, which is usually the ultimate result of a deep carious lesion. The pathogenesis of apical periodontitis is due to a non specific inflammatory

process and a specific immunologic reaction of the host in the periapical tissues: (cementum of the root, periodontal ligament and alveolar bone) in response to the infection coming from the endodontium. The establishment of this response is believed to be an attempt for the body to prevent the diffusion of the infection into the bone. With time, Apical Periodontitis causes the re-absorption of the periapical bone, its substitution with the inflammatory tissue, and the formation of a radiolucent lesion (periapical lesion). Clinical signs and symptoms associated with the different stages of apical periodontitis are represented by soft tissues swelling (periapical abscess), presence of sinus tract, pain to percussion of the tooth and to palpation of the periapical area. Yet apical periodontitis is usually a chronic infection and, in most cases, remains asymptomatic. Therefore, it is often diagnosed by the radiographic observation of a radiolucent area around the root of the affected tooth (Fig. 2). Over time, apical periodontitis may suffer an acute exacerbation and become symptomatic. Apical periodontitis can be treated by the instrumentation, disinfection and obturation of the radicular canal of the involved tooth (endodontic treatment). Unfortunately, the disease can persist or recur after treatment is completed. Then, periapical surgery or the extraction of the tooth should be considered as a final treatment (Metzger Z, Abramovitz I. In: Ingle's Endodontics 2008). Despite being different as to etiology and pathogenesis, periodontal disease and apical periodontitis show some similarities. They share a common microbiota (often Gram-negative anaerobic bacteria) (Sundqvist G 1992; Noiri Y *et al.* 2001) and are both accompanied by elevated systemic cytokine levels (Barkhordar RA *et al.* 1999; Gamonal J *et al.* 2000). Three possible metastatic pathways can be considered responsible for the consequences of oral infections on systemic diseases such as CVD:

1. metastatic spread of infection from the oral cavity, resulting from a transient bacteremia;
2. metastatic injury by circulating oral microbial toxins;
3. metastatic inflammation arising from an immune response to oral microorganisms (Thoden van Velzen SK *et al.* 1984).

In recent years, a number of reports have discussed the existence of a possible association between periodontal diseases and CVD, and have shown that these two diseases are associated in an independent way with the "classic" coronary risk factors (De Stefano F *et al.* 1993). Conversely, there are only few studies that have addressed the association between apical periodontitis and CVD.
The purpose of the present paper is to do a review of the literature on the "state of the art" of the relationship between CVD and both periodontal disease and apical periodontitis.
A MEDLINE search was conducted using the MeSH terms "cardiovascular disease (CVD)" or "atherosclerosis". A second search used the terms "infectious" and the final search combined the results of the first 2 searches and added the terms "periodontal disease" OR "apical periodontitis." Articles identified in this manner were retrieved and their reference lists searched for additional relevant articles. The search was limited to English–language publications, but no other restrictions were applied.

## ***Periodontal Disease and CVD***

Periodontal disease is one of the most common oral infections, which often affects young people: in 1995 Bochniak et al. (Bochniak M *et al.* 2004) reported that only 18.5% of the teenage population examined in their study had shown a healthy periodontal tissue. In the group aged between 35 and 44 years, the frequency of healthy periodontal tissue was reduced to 6.3%. Because of its high incidence, they concluded that periodontal disease could be counted among social diseases. Aerobic and anaerobic bacteria are the microorganisms found within the gingival pockets in periodontal disease. Streptococcus sp. and Actinomyces sp. are included among the first, while Porphyromonas gingivalis, Prevotella intermediate, Peptostreptococcus sp., Fusobacterium sp., Treponema denticola, Eikenella corrodens, and Actinobacillus actinomycetemcomitans are included among the latter (Slots J 1979; Slots J 1999). The chronic activity of bacteria, their toxins, enzymes and

metabolites, followed by a host immune response, lead to a progressive failure of periodontal attachment and premature loss of teeth.

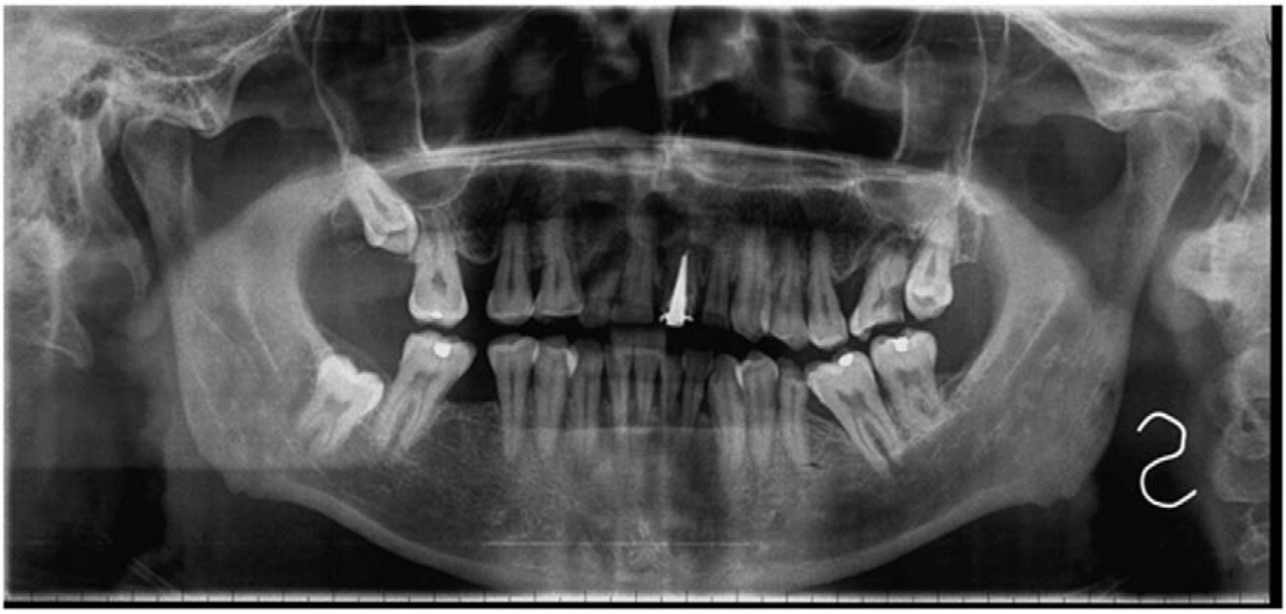

**Fig. 1. Panoramic radiograph showing a case of diffuse periodontal disease: some teeth are missing and the alveolar bone is reduced both vertically and horizontally in many sites.**

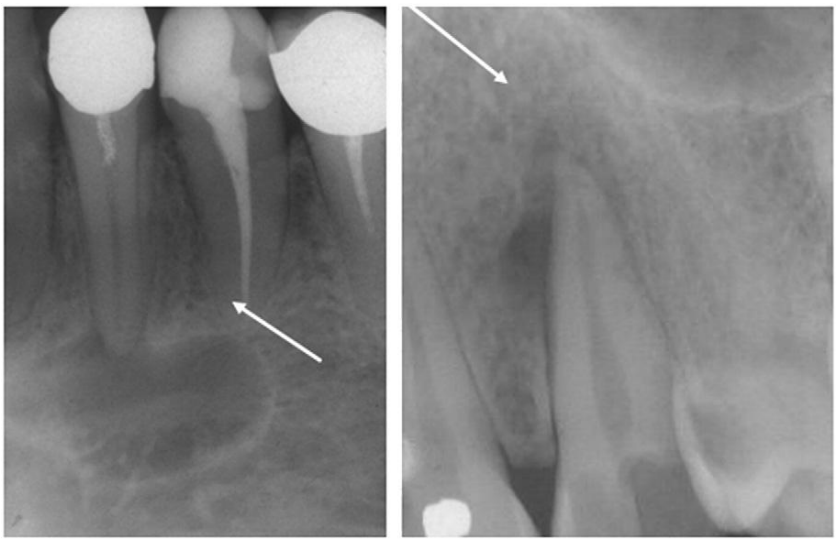

**Fig. 2. Two examples of periapical bone lesions of endodontic origin (arrowed).**

Furthermore, clinical procedures performed by dentists on the teeth and periodontium, along with the daily brushing made by patients, produce a transient bacteriemia, which may cause a secondary infection in a distant tissue or organ, including arteries. A number of epidemiologic studies have considered the possible correlation between the presence of periodontal disease and the incidence of CVD.

In 1989, Mattila et al. published a paper indicating the relationship between periodontal infections and atherosclerosis (Mattila K *et al*, 1989). They evaluated the panoramic radiographs of 100 Finnish men and women who had had a myocardial infarction and compared them with the presence and number of teeth, mouth caries, gingival and bony pockets. The conclusion was that the dental health of these patients was significantly worse than that of controls (102 subjects). The logistic regression analysis recognized periodontal disease as an independent predictor of myocardial infarction risk. De Stefano et al. (De Stefano F *et al*, 1993) realized the first prospective cohort study, based on a 14-year follow-up of 1000 subjects. The authors found a 25% increase in CHD in the patients who were clinically diagnosed with periodontal disease. This association was more pronounced in men under 50 years of age. In addition, the total mortality was higher in patients with periodontal disease and CHD than in those with CHD and healthy periodontium. That study also considered the possibility that this association could only be accidental, resulting from the combination of poor hygiene and lack of a health-conscious behavior often observed in patients with CVD. Beck et al., by using data from the Normative Aging Study (NAS) and Dental Longitudinal Study (DLS), which lasted over 30 years, studied the co-presence of tooth loss and CVD (Beck JD *et al*, 1996). They found a positive correlation between the horizontal alveolar bone loss in panoramic radiographs (indicative of periodontal disease) and the incidence of CHD. In that study, bone loss exceeding 40% was associated with a threefold increase in CHD mortality. The authors suggested that the effect of periodontal disease on systemic health is more relevant than that of smoking and other environmental CV risk factors.

The epidemiological investigation of Joshipura et al. (Joshipura KJ *et al*, 1996) evaluated CHD incidence in relation to periodontal disease and to the number of teeth present in over 70.000 healthcare professionals. That study did not find any overall associations between periodontal disease and CHD. Nevertheless, tooth loss was well correlated with increased coronary risk, especially among subjects with a positive periodontal disease history. As healthcare professionals were monitored by means of mailed questionnaires about their oral conditions, the results of that study could not be considered sufficiently reliable.

In 1997, Grau et al. (Grau AJ *et al*, 1997) compared the clinical and radiographic dental status of 166 patients with acute ischemic stroke history with that of 166 healthy controls. That study used a total dental index (TDI) reflecting caries, periapical lesions, periodontal disease and other dental lesions, and considered the low social status along with traditional risk factors for cerebrovascular ischemia. The poor dental status, as defined by TDI, resulted associated with an increased risk of ischemic cerebrovascular events. On the other hand, this correlation became less significant after the adjustment for the other CVD risk factors. Jansson et al. (Jansson L *et al*, 2001) investigated the relationship between periodontal health and fatal CVD in 1393 Swedish subjects who had already been investigated some 30 years earlier in the context of an epidemiological surveillance on dental health. Mortality rate and causes of death in the sample were registered according to death certificates. The interactional effect between dental plaque and oral health score (a sum of scores for number of missing teeth, apical lesions, caries lesions and marginal bone loss), adjusted for confounding variables, was significantly correlated to fatal coronary events. In this study, dental health proved to be a reliable risk marker for coronary death, especially in combination with smoking. A second group of studies has focused on the possible mechanisms through which periodontal disease may contribute to trigger CVD pathogenesis. These atherogenic mechanisms are basically due to: 1. the induction of platelet aggregation caused by certain oral bacteria, 2. the release of large amounts of pro-inflammatory mediators, which is the

consequence of a strong host response to oral bacterial stimulus and 3. the effect on the endothelium of bacterial and inflammatory products concentrated in the serum as a result of bacteremia. Herzberg et al. (Herzberg M *et al* 1996) evaluated the effects of oral flora on CV function in rabbits by injecting platelet-aggregating doses of 4 to 40×10 (9) cells of S. sanguis. They found that this infusion causes dose-dependent changes in blood pressure, heart rate, electrocardiogram and cardiac contractility. These changes were found consistent with the occurrence of myocardial infarction. A thrombogenic activity by S. sanguis could, therefore, justify the additional contribution of periodontal disease to CVD.

Deshpande et al. (Deshpande RG *et al*, 1998), by using an antibiotic protection assay and the transmission and scanning electron microscopy, observed the invasion of bovine and human aortic endothelial cells by Porphyromonas gingivalis. They hypothesized that this invasion could be a strategy developed by pathogens to avoid host immune response.

Haraszthy et al. (Haraszthy V *et al*, 2000) examined 50 human specimens obtained during carotid endarterectomy for the presence of Chlamydia pneumoniae, human Cytomegalovirus (HCMV), and bacterial 16S rRNA using PCR assays. Thirty-eight percent were positive for HCMV and 18% for Chlamydia pneumoniae. PCR assays for bacterial 16S rRNA also indicated the presence of bacteria in 72% of the surgical samples. Subsequent hybridization of the bacterial 16S rRNA positive specimens with species-specific oligonucleotide probes revealed that 44% of the 50 atheromas were positive for at least one of the target periodontal pathogens (B. forsythus, 30%; P. gingivalis, 26%; A. actinomycetemcomitans, 18%; P. intermedia, 14%). Fifty-nine percent of periodontal pathogen-positive surgical specimens were positive for 2 or more of the target species. The authors concluded that periodontal pathogens, present in atherosclerotic plaques, may influence the development and progression of atherosclerosis. Kuramitsu et al. (Kuramitsu HK *et al*, 2001) demonstrated that P. gingivalis shows several properties that could play a role in CVD as mediators of LDL oxidation, foam cell formation, and atherosclerotic plaque rupture. In the Atherosclerosis Risk in Communities Study (ARIC),

which involved 6000 patients, Beck et al. (Beck JD *et al*, 2001) reported an association between periodontal disease severity and the intimate/media thickness of carotid artery. Subsequent analysis of data collected over a period of 25–30 years in NAS and DLS studies indicated that periodontal disease was a significant risk predictor for peripheral arterial disease. The study by Persson et al. (Persson RE *et al*, 2002) demonstrated an association between alveolar bone loss and increase in calcium deposit within the wall of the internal carotid artery. Samples of aorta were taken from patients undergoing open-heart surgery to investigate the presence of periodontal pathogens by PCR and subsequent hybridization (Stelzel M *et al*, 2002). Bacterial DNA was found in 23 of 26 (88.5%) samples, in most cases only in concentrations around the detection limit. Four samples were clearly positive for Porphyromonas gingivalis. The authors emphasized the possible connection between periodontal pathogens entering the CV system and CVD. D'Aiuto et al. (D'Aiuto F *et al*, 2004) examined the outcomes of periodontal therapy in terms of changes in C-reactive protein (CRP)-associated CV risk. Serum inflammatory responses [interleukin-6 (IL-6) and CRP] were monitored 2 and 6 months after non-surgical periodontal treatment in 94 healthy subjects suffering from severe periodontitis. At 6-month control, patients who had a better oral response to periodontal therapy were more likely to have decreased their inflammatory risk category after correcting for age, gender, ethnicity and cigarette smoking. Moreover, a significant decrease in number of subjects with a medium and high CRP-associated risk was observed. The authors concluded that "periodontitis may add to the systemic inflammatory burden of the individual and may result in increased levels of CV risk based on serum CRP concentrations". In 2005, Cavrini et al. (Cavrini F *et al*, 2005) reported two cases of patients affected by hypertension and atherosclerotic lesions. Porphyromonas gingivalis and Treponema denticola were identified by PCR and FISH in atheromatous plaques in both patients. Obviously, those 2 cases alone do not allow a definitive conclusion on the correlation between periodontal disease and atherosclerosis.

## ***Conclusions on the Correlation between Periodontal Disease and CVD***

A series of epidemiological studies (De Stefano F *et al*, 1993; Bochniak M *et al*, 2004; Mattila K *et al*, 1989; Beck JD *et al*, 1996; Joshipura KJ *et al*, 1996; Grau AJ *et al*, 1997; Jansson L *et al*, 2001) has suggested that periodontal disease may contribute to the genesis of CVD; observational studies (Herzberg M *et al*, 1996; Deshpande RG *et al*, 1998; Haraszthy V *et al*, 2000; Kuramitsu HK *et al*, 2001; Beck JD *et al*, 2001; Persson RE *et al*, 2002; Stelzel M *et al*, 2002; D'Aiuto F *et al*, 2004; Cavrini F *et al*, 2005), for their part, have addressed the mechanisms by which periodontal disease might influence the development of CVD. This association has been hypothesized to be attributable to a common inflammatory response trait, which exposes individuals to the development of both periodontal disease and atherosclerosis. Furthermore, periodontal disease is believed to provide a "biological burden" of inflammatory cytokines, which promote atherosclerosis and thrombotic events. However, it should be noted that epidemiologic research cannot identify the cause: periodontal disease may occur together with some forms of CVD or represent an oral manifestation of the same disease. Future research should be aimed at determining whether periodontal disease can directly damage the CV system.

## ***Apical Periodontitis and CVD***

Apical periodontitis is a sequel to endodontic infection and develops as the host response to microbial infection that comes from the root canal system of the affected tooth (Nair PRN, 2004). Endodontic infection that leads to apical periodontitis is caused by a mixture of oral bacterial species also found in dental plaque, dominated by obligate anaerobes (most frequently Peptostreptococcus, Eubacterium, Prevotella, Porphiromonas, Fusobacterium,

Streptococcus) (Baumgartner JC *et al*. Microbiology of Endodontic Disease. In Ingle's Endodontics 2008).

Only a few studies have investigated the possible correlation between pulpal inflammation and/or apical periodontitis and CVD. An association has been noted between apical periodontitis and stroke, as well as between a "composite status of oral health" (caries, periapical lesions, number of endodontically treated teeth) and CVD (Caplan DJ *et al*, 2006). This section can be further divided into two parts 1. the consequences of dental procedures performed to treat endodontic infections on systemic diseases, and 2. the association of apical periodontitis and CVD.

## ***Association of Apical Periodontitis and CVD: Consequences of Dental Procedures Performed to Treat Endodontic Infections on Systemic Diseases***

In 1994, Debelian (Debelian GJ *et al*, 1994) observed that human periodontal and endodontic infections were associated with complex microfloras: approximately 350 bacterial species were identified in marginal periodontitis and other 150 were found in apical periodontitis. Both groups of bacteria mainly included anaerobic pathogens, among which gram-negative rods were the most frequently isolated. The proximity to the bloodstream of microflora present in the root canal and periapical tissues can cause a transient bacteremia during clinical dental procedures (e.g., tooth extraction, periodontal and endodontic treatments). Normally, microorganisms penetrated into the bloodstream are eliminated by the host within minutes. However, it is known that in patients with valvular heart disease or vascular diseases, a transient bacteremia may lead to infective endocarditis and myocardial or cerebral infarction. Other forms of systemic disease, such as brain abscesses, hematological and implant infections, have also been correlated with oral microorganisms (American Association of Endodontists, 2000). It is thus evident that both endodontic surgical procedures and non-surgical instrumentation of root canals during endodontic treatments can produce a

transient bacteremia. One study on bacteremia in conjunction with endodontic therapy was conducted on blood samples taken from the patients during and 10 minutes after root canal instrumentation (Debelian GJ *et al*, 1995). Bacteremia occurred in 54% of patients when teeth were deliberately instrumented 2 mm beyond the apical foramen and in 31% of patients when instrumentation ended inside the root canal 1 mm short of the apical foramen. Biochemical tests and antibiograms showed that microorganisms isolated from both the tooth root canal and the bloodstream had the same profiles in each patient. In a subsequent report, bacteremia with predominance of anaerobes was detected in 30% of patients following endodontic treatment (Savarrio L *et al*, 2005). We must bear in mind that a tooth extraction causes bacteremia in 100% of times (Heimdahl A *et al*, 1990) and that the risk to acquire bacterial endocarditis from a dental treatment ranges from1 / 100,000 in patients with previous endocarditis to 1/amillion in those with mitral valve prolapse and regurgitation (Pallasch TJ, 2003).

## ***Apical Periodontitis (Endodontic Infection) and CHD***

In 2003, Frisk et al. (Frisk F *et al*, 2003) published the first cross-sectional study that examined the possible association between various components of endodontic disease and CHD. A connection between dental infections, probable cause of vascular abnormalities, and the genesis of atherosclerosis was hypothesized. The study was conducted in Goteborg in 1992–93, on a representative sample of women (n=1056) aged between 38 and 84 years. The dependent variable was CHD [i.e. angina pectoris and/or a history of myocardial infarction (n=106)]. The independent variables were: number of root-filled teeth, number of teeth with periapical lesions (as radiolucencies seen in the radiographs), tooth loss, age, marital status, smoking, alcohol habits, waist/hip ratio, serum cholesterol and triglyceride concentrations, hypertension and diabetes. By using the multivariate logistic regression analysis, researchers could not prove that endodontic variableswere predictive of CHD. Only age and tooth loss were significantly associated with

CHD [OR=1.07 (CI=1.03–1.12) and OR=2.70 (CI=1.49–4.87), respectively]. The bivariate logistic regression analysis showed a significant association between endodontically treated teeth and CHD; conversely, the same analysis did not support any associations between periapical lesions and CHD.

In 2006, Caplan et al. (Caplan DJ *et al*, 2006) reported the results of the VA Dental Longitudinal Study, in which 708 participants (all males, mean age 47.4 years) were recruited. In accordance with the evidence that periodontal disease is more manifest in young male patients (Bochniak M *et al*, 2004), the authors hypothesized that also young men with a greater number of endodontic lesions might be more prone to develop CHD. Patients underwent comprehensive medical and dental examinations (including panoramic radiographs) at baseline and every three years for up to 32 years (median 24 years). Cox regression models estimated the relationship between incident lesions of endodontic origin and time to CHD diagnosis. Thirty-five percent of all participants had at least 1 periapical lesion and 23.4% of them were subsequently diagnosed with CHD. Twenty-seven percent of participants aged ≤45 years and 41% of those aged N45 years had one or more periapical lesions. Among the subjects who were subsequently diagnosed with CHD, the youngest showed a greater number of apical lesions compared to older people. Among participants aged 40 years or younger, incident lesions of endodontic origin were significantly associated with time to CHD diagnosis, after adjustment for covariates of interest, with hazard ratios decreasing as age increased. Among participants aged N40 years, no statistically significant associations were observed.

Following these results, the authors (Caplan DJ, 2004) asserted that the "*mechanisms linking endodontic disease to CHD risk might be similar to those hypothesized for the associations between periodontal disease and CHD, where a localized inflammatory response to bacterial infection leads to the release of cytokines into systemic circulation, with subsequent deleterious vascular effects*" (Caplan DJ *et al*, 2002; Caplan DJ, 2004). In accordance with the above assertion, the interdependence between CVD and endodontic infection could be demonstrated by the observation that also endodontic disease is

produced by gram-negative anaerobes (Baumgartner JC, 1991; Sundqvist G, 1992) and characterized by the release of cytokines (Miller GA *et al*, 1996; Kuo ML *et al*, 1998) and high levels of inflammatory mediators (Marton I *et al*, 1988; Marton IJ *et al*, 1992). Caplan et al. also explained that this association proves to be more effective among young people since, with time, older subjects may develop other characteristics more strongly associated with CHD pathogenesis. Alternatively, the explanation may lie in the "healthy survivor" phenomenon, meaning that older people tend to be healthier than other members of the same cohort who die before. Using data from the Health Professionals Follow-Up Study (HPFS), with its large cohort of 34,683 participants, Joshipura et al. (Joshipura KJ *et al*, 2006) evaluated the connection between pulpal inflammation and incidence of CHD. The hypothesis to be tested was that pulpal inflammation may lead to increased CHD risk. As an indicator of pulpal inflammation, the presence of one or more root canal therapy (RCT) was used. Participants were all male health professionals aged 40 to 75 years who had drawn every two years a mailed questionnaire concerning their health conditions. Individuals with prior CVD or diabetes were excluded. A significant correlation between RCT and CHD incidence was limited to the subgroup of dentists. Since the measure of pulpal inflammation was based on self-reported RCT, it is not known whether RCT were performed in response to a pulpal inflammation/ infection. Moreover, the proportion of RCT related apical periodontitis is not known. One hypothesis for this outcome may be that dentists are less likely to submit themselves to root canal treatment if there is no diagnostic evidence of pulpal inflammation/infection. In accordance with some previous studies (Grau AJ *et al*, 1997; Jansson L *et al*, 2001), the application of multivariate analysis to data from HPFS showed that dental caries were not associated with CHD and suggest a possible modest association between the latter and pulpal inflammation. These results could be explained as an actual lack of biologic interdependence, or as a failure of the questionnaire to distinguish from active caries and restorations, or by the fact that only deep caries may influence the patient's health status. In a summary thesis from 2007 (Frisk F, 2007), Frisk mixed data from the above mentioned

2003 study (Frisk F *et al*, 2003) with the results of 2 epidemiological studies on apical periodontitis in the Swedish population (Frisk F *et al*, 2005; Frisk F *et al*, 2006). The objectives of this thesis were to further clarify clinical and socioeconomic risk factors for apical periodontitis and to reconsider, in a larger population, the possible association between apical periodontitis and CHD. In the Population Study of Women in Göteborg, participants aged 38–84 years were recruited for cross-sectional and longitudinal analysis of endodontic status over 24 years. A cross sectional sample was used for exploring associations between apical periodontitis or socio-economic risk factors and CHD in multivariate logistic regression models. In the Population Study on Oral Health in Jönköping, random samples of women aged 20–70 years were used. Apical periodontitis was radiographically recorded and the root filling quality was assessed with respect to length and seal. Inadequate root filling quality was predictive of apical periodontitis with a 4.5 OR. On the other hand, the results did not reveal any significant associations between apical periodontitis and CHD nor between socio-economic risk factors and apical periodontitis. In a recent study, Caplan et al. (Caplan DJ *et al*, 2009) evaluated the correlation between self-reported history of endodontic therapy (ET) and CHD prevalence. To that end, they used data derived from oral health questionnaires, medical evaluations and clinical dental examinations of 6.651 dentate participants in the Atherosclerosis Risk in Communities Study. Final multivariable regression models indicated that, among participants with 25 or more teeth, those who reported having undergone two or more ET showed a significantly higher prevalence of CHD than those reporting no history of ET. Among participants with 24 or fewer teeth, no significant differences in CHD prevalence were observed among groups, regardless of their ET history.

## ***Conclusions on The Correlation Between Apical Periodontitis and CVD***

While the deep connections between periodontal disease and CVD have been well documented by several studies, the potential CV consequences of apical periodontitis/endodontic disease remain largely unknown and controversial. The issue has been addressed only recently and has produced mixed results, with studies in favor of a positive correlation between apical periodontitis and coronary risk (Caplan DJ *et al*, 2006; Caplan DJ *et al*, 2009), and other negative (Frisk F *et al*, 2003; Frisk F, 2007) or inconclusive (Joshipura KJ *et al*, 2006). Unfortunately, the necessary scientific rigorousness was not always applied for a better understanding of the relationship between the presence of periapical lesions and CV risk. In trials that have followed each other over time, weak surrogate parameters of risk have been used, several populations of different ages, difficult to compare, were studied and control groups have not been provided at any time. Apical periodontitis is widely present in endodontically treated teeth and is often associated with a poor quality endodontic treatment (Caplan DJ, 2004). Therefore, it is always more difficult to evaluate the "cumulative endodontic infectious burden" for average patients (Caplan DJ *et al*, 2006). On the basis of the still equivocal suggestions of literature (Table 1) we should feel encouraged to better investigate this issue. A more precise understanding of the connection between endodontic infection and inflammation and CV risk would be of great interest not only from a scientific point of view but also from a public health perspective (American Association of Endodontists. 2000; Caplan DJ *et al*, 2009). It is therefore extremely urgent to know whether apical periodontitis represents only the oral component of a systemic disease, or shares with it a common etiology. Only a more focused and rigorous scientific research can determine a definitive opinion on the relationship between endodontic disease and CVD. Therefore it would be important to use dental infection as an independent variable in future CVD research.

**Table 1. List of the epidemiologic studies which investigated the association between Apical Periodontitis (AP) and Coronary Heart Disease (CHD)**

| Authors, year | Participants | Aim | Conclusions |
|---|---|---|---|
| Frisk et al. [47] | 1056 women participants | Possible association between endodontic treatment or AP and CHD | By multivariate logistic regression: no association between endodontic variables and CHD. By bivariate logistic regression: association between endodontically treated teeth and CHD; no association between teeth with AP and CHD |
| Caplan et al. [40] | 708 male participants in the VADLSa | Possible link between number of lesions of AP and CHD | Higher relationship between AP and development of CHD (especially in young age group) |
| Joshipura et al. [55] | 34,683 participants from HPFSb | Possible association between pulpal inflammation (endodontic treatment) and incidence of CHD | Strong association between a positive self-reported history of Endodontic Treatment incidence of CHD |
| Frisk [58] | 3499 women participants from the PSWGc and random samples of dentate individuals (n 2066) aged 20–70 years from the PSOHJd | Endodontic status in Swedish populations and possible association between AP and CHD | No significant association between AP and CHD and socio-economic risk factors and AP respectively. |
| Caplan [59] | 6651 participants from the ARICe | Possible association between endodontic disease (Endodontic Treatment) and CHD | Association of self-reported history of ET among participants with 25 or more teeth, with CHD |

a Veterans Affairs Dental Longitudinal Study.
b Health Professionals Follow-Up Study.
c Prospective Study of Women in Göteborg, Sweden.
d Population Study on Oral health in Jönköping, Sweden
e Atherosclerosis Risk in Communities.

# Chapter 3
# AP & CVD

Forty men between the ages of 20 and 40 years who were free from periodontal disease, CVD, and traditional CV risk factors were enrolled in the study (Cotti E *et al*, 2011); 20 subjects had AP, and 20 acted as controls. All subjects underwent dental examination and complete cardiac assessment: physical examination, electrocardiogram, conventional and tissue Doppler echocardiography, and measurement of endothelial flow reserve (EFR). The following laboratory parameters were tested: interleukins -1, -2, and -6 (IL-1, IL-2, IL-6), tumor necrosis factor alpha, and asymmetrical dimethylarginine (ADMA). Data were analyzed by using the 2-tailed Student's t test, Pearson t test (or Spearman t test for nonparametric variables), and multivariate linear regression analysis.

Results: Echocardiography revealed no abnormalities in any of the subjects studied. ADMA levels were inversely correlated with EFR ($P < .05$) and directly correlated with IL-2 ($P < .001$). Patients with AP presented with significantly greater blood concentrations of IL-1 ($P < .05$), IL-2 ($P < .01$), IL-6 ($P < .05$), and ADMA ($P < .05$) and a significant reduction of EFR ($P < .05$).

Conclusions: Increased ADMA levels and their relationship with poor EFR and increased IL-2 might suggest the existence of an early endothelial dysfunction in young adults with AP. It is an acknowledged fact that the equilibrium in the circulatory system is maintained by normal functional endothelium that, by inhibiting platelet aggregation, monocyte adhesion, and vascular smooth muscle cell proliferation, maintains the physiological balance between procoagulant and anticoagulant forces (Flammer AJ *et al*, 2010). Cardiovascular diseases (CVDs) are known to originate from endothelial inflammatory dysfunction (Muller MM, Griesmacher A, 2000) and are influenced by well-known CV risk factors, including smoking, diabetes, hypertension, and dyslipidemia (Batsis JA, Lopez-Jimenez F, 2010).

Chronic inflammation plays a crucial role in the pathogenesis and progression of atherosclerosis and at the same time promotes acute CV events such as plaque rupture and coronary thrombosis (Ross R, 1999).

In recent years, a number of studies have demonstrated the possible association between CVD and periodontal disease (Niedzielska I *et al*, 2008). Apical periodontitis (AP) is an inflammatory and frequently chronic disorder of the periapical tissues caused by the persistence of a microbial infection within the endodontic system of the affected tooth (Metzger Z *et al*, Periapical lesions of endodontic origin. In Ingle's endodontics, 2008; Nair PNP. In: Cohen S, Hargreaves K, eds. 2006). The scientific literature has failed to provide unequivocal interpretation of the potential connection between endodontic infection and CV risk (De Stefano F *et al*, 1993). Therefore, it would be of great interest, both from a scientific point of view and from a public health perspective, to ascertain whether the presence of AP is associated with indices of CV function and biological markers associated with systemic inflammation.
A prospective study was designed to investigate whether the state of low-grade chronic inflammation that accompanies AP is capable of significantly altering cardiac endothelial function. In the initial part of the study the first 40 subjects, 20 with AP and 20 controls, all of whom were men younger than the age of 40 and were free from CVD and CV risk factors, were studied. In future studies the sample size will be increased, and evaluation will be extended to women and older people.

## ***Study Design and Patient Population***

The present study represents the first part of an observational cross-sectional trial conducted on young men observed at the Diagnostic Section of the Dental Clinic at the University of Cagliari during 2009. Subjects were divided into 2 groups, patients affected by AP and healthy controls. The study protocol was approved by the Institutional Ethics Committee (Azienda

Ospedaliero-Universitaria, University of Cagliari). Written informed consent was obtained from all subjects.

**Inclusion Criteria Were the Following:**

1. Male patients
2. Age 20–40 years
3. Presence of at least 1 radiographically assessed endodontic lesion
4. Presence of at least 25 teeth
5. Echocardiographic left ventricle ejection fraction (LVEF) value ≥55%
6. Hepatic and renal function within normal limits (bilirubin ≤1.5 mg/dL, creatinine≤2.0 mg/dL)

**Exclusion Criteria Were as Follows:**

1. Presence of localized or diffuse periodontal disease
2. Presence of non-endodontic lesions in the maxillary bones
3. Presence of CV risk factors (arterial hypertension, dyslipidemia, diabetes mellitus, obesity, history of smoking)
4. LV hypertrophy at echocardiography
5. Previous and/or current CV or cerebrovascular disease
6. Presence of chronic inflammatory conditions in other districts involving systemic health

Forty consecutive patients who registered for a dental check-up at the School of Dentistry were enrolled; 20 were affected by AP (age_standard deviation [SD], 35_5 years; range, 22–40 years), and 20 were control subjects matched for age and physical characteristics (27_3 years; range, 21–33 years). All patients underwent a complete CV assessment: medical history, physical examination, blood pressure measurement, 12-lead electrocardiogram, and conventional and tissue Doppler (TDI) echocardiographic analysis. In addition, blood samples were collected for the assessment of circulating levels of

interleukin-1, -2, and -6 (IL-1, IL-2, IL-6), tumor necrosis factor alpha (TNF-a), and asymmetrical dimethylarginine (ADMA).

***Dental Examination***

A complete dental examination was performed on each patient. A panoramic radiograph was examined and used as initial screening and was followed by selected periapical radiographs taken on teeth suspected of presenting AP and in all teeth with root canal treatments or presenting extensive restorations (including prosthetic restorations) with or without previous endodontic treatment. Periapical radiographs were taken with a radiographic unit (Castellini, Castelmaggiore, Italy) by using a long cone paralleling technique (70 Kv; 10 Ma; film-focus distance, 28 cm) and Ultra Speed film (Eastman Kodak, Rochester, NY). Radiographs were examined in a darkened room by an expert endodontist by using an illuminated viewer box with 3.5_ magnification to assess the presence of a periapical lesion involving at least 1 root of a given tooth. To reduce interobserver variability all radiographs were randomly evaluated by a second expert observer, and an average of each observation was calculated.

Intraoral examination was then completed. By using both radiographic and intraoral evaluations the following parameters were recorded:

1. Number of teeth present
2. Number and location of restored teeth
3. Number of endodontically treated teeth
4. Number and location of teeth affected by carious processes
5. Soft tissue assessment (presence and location of swelling/sinus tracts)
6. Periodontal probing
7. Number and location of teeth with AP
8. State of the upper and lower jaws

## Conventional and TDI Echocardiography

Echocardiographic images were recorded by using a commercially available system equipped with TDI and SR imaging (Toshiba APLIO CV ultrasound system-SSA 770A/CV; Toshiba Corp, Tochigi, Japan). LVEF was obtained from the apical 4- and 2-chamber views according to Simpson's rule and was considered abnormal when less than 55%. Pulsed wave Doppler examination of the LV inflow from the 4- chamber view was performed with the sample volume placed between the mitral leaflet tips and early (E) and late (A) diastolic peak velocities; E deceleration time (DecT) was measured, and E/A ratio was subsequently derived (9). Longitudinal function was evaluated by using pulsed TDI at the mitral annulus, placing the sample volume in the basal segment of the interventricular septum from the apical 4-chamber view; peak velocities in systole, isovolumic relaxation time, and early and late diastole were measured. For more accurate measurements TDI curves were obtained from raw data analysis. LV longitudinal function was evaluated from raw data; myocardial Strain and Strain rate were also quantified in the interventricular septum. All examinations were performed by the same experienced echocardiographer who was unaware of patients' clinical status and therapeutic regimen. A simultaneous electrocardiographic tracing was also obtained. To reduce interobserver variability, all echocardiographic data were randomly read by a second experienced observer, and an average value for each measurement was calculated. Reproducibility of TDI parameters in our laboratory had been previously documented (Zoncu S *et al*, 2002).

## Inflammatory Stress Markers

Blood samples were collected in tubes with clot activating factors and centrifuged immediately after collection; serum was stored at _20_C until assay. Levels of IL-1, IL-2, IL-6, and TNF-a were determined by enzyme-linked immunosorbent assay (Immunotech, Marseille, France). Results are expressed in picograms per milliliter.

Plasma ADMA was analyzed by high-performance liquid chromatography coupled with laser-induced fluorescence detection. In all subjects a blood sample was obtained by venipuncture of the antecubital vein at 8 AM after overnight fasting. Blood samples were collected into serum tubes, centrifuged, and extracted by serum protein electrophoresis at room temperature. After derivatization, ADMA was quantified by the chromatographic method.

**Endothelial Function**

Endothelial flow reserve (EFR) was measured at the level of the distal extremity in the upper limbs by means of peripheral arterial tonometry (PAT), a non–operator-dependent method providing a reproducible index of endothelial-dependent vasodilation (Rubinshtein R *et al*, 2010). We used the ENDO-PAT2000 model (Itamar Medical, Caesarea, Israel), an apparatus measuring changes to vasal tone influenced by the endothelium in the fingers through biosensors. Modifications of vasal tone are produced by occlusion of the brachial artery for 5 minutes with consequent hyperemic response; the contralateral arm was used as control.

**Statistical Analysis**

Data are reported as mean ± SD. Differences between values assessed in AP patients and healthy controls were calculated by the Student's 2-tailed t test for unpaired data. Correlation between instrumental (EFR) and laboratory variables was assessed by Pearson t test. P values were considered as significant when $P \leq 05$.

**Results**

Table 1 summarizes the basic dental features of the patients in the study group (AP).

In case of multirooted teeth, they were simply classified as having AP whether they exhibited 1 or more periapical lesions. There were no appreciable differences in anthropometric, clinical, and chemical parameters between AP subjects and controls. All patients in the study sample presented at least 1

lesion of endodontic origin. Neither electrocardiogram nor standard and TDI echocardiographic examinations performed at baseline revealed abnormalities in any of the subjects studied (Table 2).

A significant direct correlation between ADMA and IL-2 increases ($r = 0.680$, $P < .001$) (Fig. 1) and an inverse correlation between ADMA and EFR ($r = \_0.3704$, $P < .02$) (Fig. 2) were observed.

Patients with AP displayed statistically higher serum levels of IL-1 ($P < .05$), IL-2 ($P < .01$), and IL-6 ($P < .05$) (Fig. 3), whereas TNF did not show significant differences between the 2 groups (17.8 _ 7.0 pg/mL versus 16.5 _ 11.9 pg/mL; $P < .32$). In addition, plasma ADMA levels were significantly higher in patients with AP, compared

with the control group (0.73 _ 0.14 mmol/L versus 0.65 _ 0.09 mmol/L, $P < .05$) (Fig. 4). The mean value of endothelial reserve, measured as EFR, in the study sample was at the lower limit of the normal range and significantly lower than that detected in controls (2.08 _ 0.3 versus 2.4 _ 0.5, $P <.01$) (Fig. 4).

**TABLE 1.** Dental Status in Patients with AP

| | Patients | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| Localization of periapical lesions | #19, #18, #16 | #30, #14, #19 | #19, #8 | #12 | #5, #6, #18 | #1, #7, #15, #32 | #19, #30 | #2, #5 | #19, #14, #18, #30 | #11 | #14 | #15 | #14, #3 | #8 | #19 | #17, #32, #10 | #30 | #19 | #12, #15, #29, #30, #31 | #18, #19 |
| No. of periapical lesions | 3 | 3 | 2 | 1 | 3 | 4 | 2 | 2 | 4 | 1 | 1 | 1 | 2 | 1 | 1 | 3 | 1 | 1 | 5 | 2 |
| No. of caries and important restorations | 6 | 12 | 2 | 19 | 15 | 14 | 14 | 8 | 18 | 12 | 4 | 5 | 11 | 5 | 6 | 14 | 4 | 1 | 16 | 11 |
| No. of root-filled teeth | 2 | 5 | 1 | 4 | 5 | 5 | 5 | 1 | 9 | 4 | 1 | 2 | 2 | 1 | 1 | 7 | 3 | 0 | 7 | 5 |
| No. of teeth untreated | 25 | 19 | 28 | 7 | 14 | 8 | 14 | 20 | 14 | 6 | 28 | 27 | 20 | 25 | 23 | 5 | 24 | 31 | 14 | 21 |
| No. of teeth present | 31 | 31 | 29 | 26 | 29 | 22 | 28 | 28 | 32 | 18 | 32 | 32 | 31 | 30 | 29 | 19 | 28 | 32 | 30 | 32 |
| Age (y) | 31 | 38 | 40 | 40 | 39 | 40 | 40 | 39 | 37 | 35 | 32 | 35 | 30 | 30 | 32 | 40 | 35 | 22 | 40 | 32 |

**TABLE 1. DENTAL STATUS IN PATIENS WITH AP**

**TABLE**

**TABLE 2. Standard and TDI Echocardiographic Examinations in Both Groups**

| Parameters | Healthy subjects | AP patients | P value |
|---|---|---|---|
| E (cm/s) | 97.52 _ 9.02 | 94.5 _ 7.20 | .29 |
| A (cm/s) | 64.9 _ 11.13 | 66.2 _ 12.16 | .74 |
| E/A | 1.55 _ 0.35 | 1.47 _ 0.29 | .53 |
| E t (cm/s) | -12.1 _ 1.29 | _12.01 _ 1.32 | .85 |
| A t (cm/s) | -7.36 _ 1.33 | -7.33 _ 1.08 | .96 |
| E/A t | t 1.71 _ 0.36 | 1.65 _ 0.26 | 57 |
| S | 7.55 _ 0.68 | 7.49 _ 0.68 | .77 |
| Strain | 1.87 _ 0.15 | 1.81 _ 0.16 | .22 |
| Strain rate | 19.85 _ 1.14 | 19.6 _ 1.23 | .51 |

**A, late peak velocity-pulse Doppler (cm/second); A t, early peak velocity-tissue Doppler (cm/second); E, early peak velocity-pulse Doppler (cm/second); E/A, early/late peak velocity ratio; E t, early peak velocity-tissue Doppler (cm/second); S, systolic wave-tissue Doppler imaging (cm/second).**

# Chapter 4

## ***DISCUSSION***

In our study we found (1) a direct correlation between IL-2 and ADMA increases; (2) an inverse correlation between ADMA and EFR;(3) a significant increase in serum levels of IL-1, IL-2, IL-6, and ADMA in patients with AP; and (4) EFR values in the lower limit of normal in AP group, significantly reduced when compared with healthy controls. Both groups featured a lack of clinical and preclinical cardiac injury, as evidenced by normal parameters observed at conventional and TDI echocardiography.

Epidemiologic studies (De Stefano F *et al*, 1993; Bochniak M *et al*, 2004; Beck JD *et al*, 1996; Jansson L *et al*, 2001) and observational studies (Deshpande RG *et al*, 1998; Haraszthy V *et al*, 2000; Beck JD *et al*, 2001; Stelzel M *et al*, 2002; D'Aiuto F *et al*, 2004; Cavrini F *et al*, 2005) have suggested that periodontal disease might contribute toward the genesis of CVD, in line with the hypothesis that a common inflammatory response trait might expose individuals to the development of both periodontal disease and atherosclerosis. On the contrary, the potential CV consequences of AP (taken as the independent variable) are still largely unknown and remain controversial; the issue has only recently been addressed, with some studies reporting a positive correlation between AP and coronary risk (Caplan DJ *et al*, 2006; Caplan DJ *et al*, 2009) and others reporting negative (Frisk F *et al*, 2003; Frisk F, 2007) or inconclusive (Joshipura KJ *et al*, 2006) findings. However, epidemiologic research alone is not sufficient to identify the cause of a morbid phenomenon; it would only be possible to suggest a potential association of AP with specific forms of CVD. Recently, Segura-Egea et al (Segura-Egea JJ *et al*, 2010) investigated the prevalence of AP and endodontic treatment in hypertensive patients and failed to find any association between the 2 pathologic conditions.

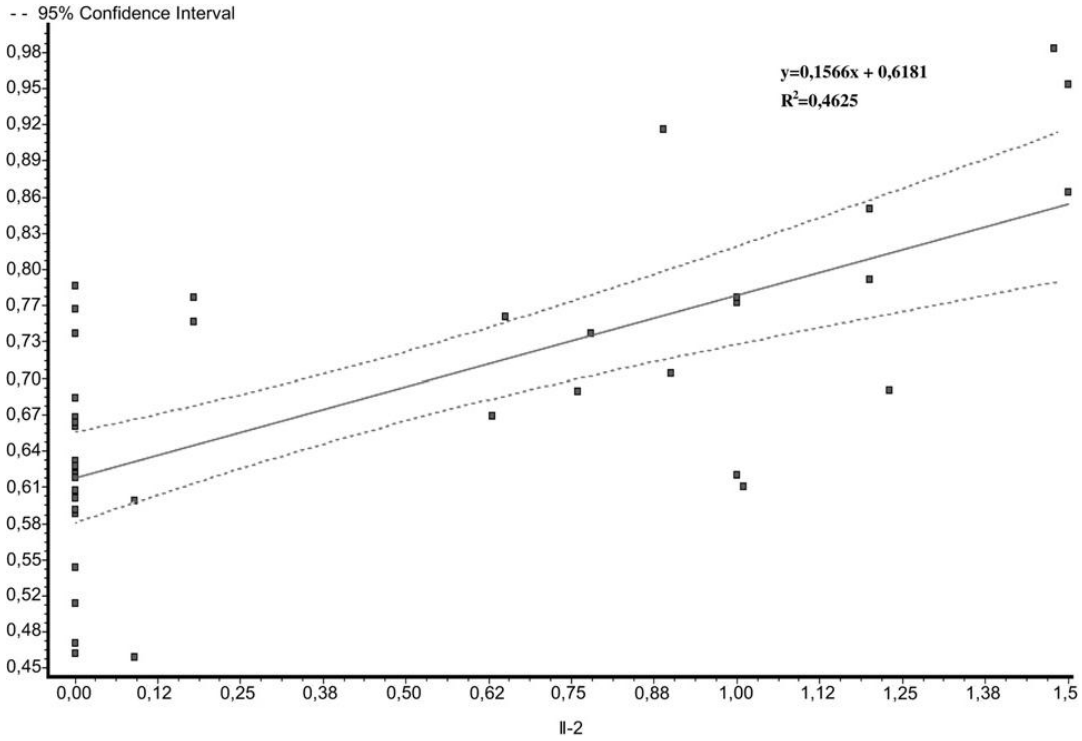


**Figure 1. Correlation between IL-2 and ADMA**

One of the aims of the present study was to overcome the limitations posed by previous studies by selecting a population of young adult male patients (20–40 years) with AP and devoid of CV risk factors and other diseases. In addition, a control group comprising male individuals of the same age and physical characteristics as patients who had no AP were assessed. Female subjects were excluded from the study to avoid the confounding factor represented by estrogen protection on the cardiovascular system in women of childbearing age. To identify early changes in the myocardial function of the young patients studied, TDI, a more reliable technique than conventional Doppler in the evaluation of LV diastolic performance, was used (Angelo LC *et al*, 2010). Moreover, TDI is capable of demonstrating changes in the regional function that are not revealed by global LVEF (Greenberg NL *et al*, 2002). Application of the abovementioned echocardiographic approach allowed us to rule out the presence of initial cardiac abnormalities in patients with AP.

In the biological background for the development of CVD, a lowgrade chronic inflammation might play a role as a risk factor for development of atherosclerosis because of its ability to induce endothelial dysfunction. IL-6 is a multifunctional cytokine playing a central role in inflammation and tissue

injury. Its levels positively correlate with higher all-cause mortality, unstable angina, LV dysfunction, propensity to diabetes and its complications, hypertension, and obesity (Tracy RP, 1999; Sarwar N *et al*, 2009).

Moreover, proinflammatory cytokines were revealed to be sensitive systemic markers of tissue damage and predictive of future cardiac adverse events among apparently healthy men (Ridker PM *et al*, 2000). Moreover, IL-2 is a cytokine produced by T-helper 1 cells involved in B-cell activation, macrophage stimulation, and natural killer cell and T-cell proliferation (Wang J *et al*, 2009). In addition, IL-2 is regarded as a proinflammatory cytokine (Hoyer KK *et al*, 2008; Lan RY *et al*, 2008). AP is a prevalently chronic infection affecting the teeth and maxillary bones caused by gram-negative anaerobic microbiota (Metzger Z *et al*, Periapical Lesions of Endodontic Origin. In: Ingle's Endodontics. 2008; Nair PNP. In: Cohen S, Hargreaves K, eds. 2006). Elevated levels of inflammatory mediators and increased cytokine concentrations have been detected in periapical tissues of teeth with AP (Metzger Z, Abramovitz I. Periapical Lesions of Endodontic Origin. In: Ingle's Endodontics. 2008; Nair PNP. In: Cohen S, Hargreaves K, eds. 2006; Barkhordar RA *et al*, 1999). IL-6 and TNF-a levels have been shown in periapical lesions and in the liver of rats with induced periapical abscesses (Bain JL *et al*, 2009).

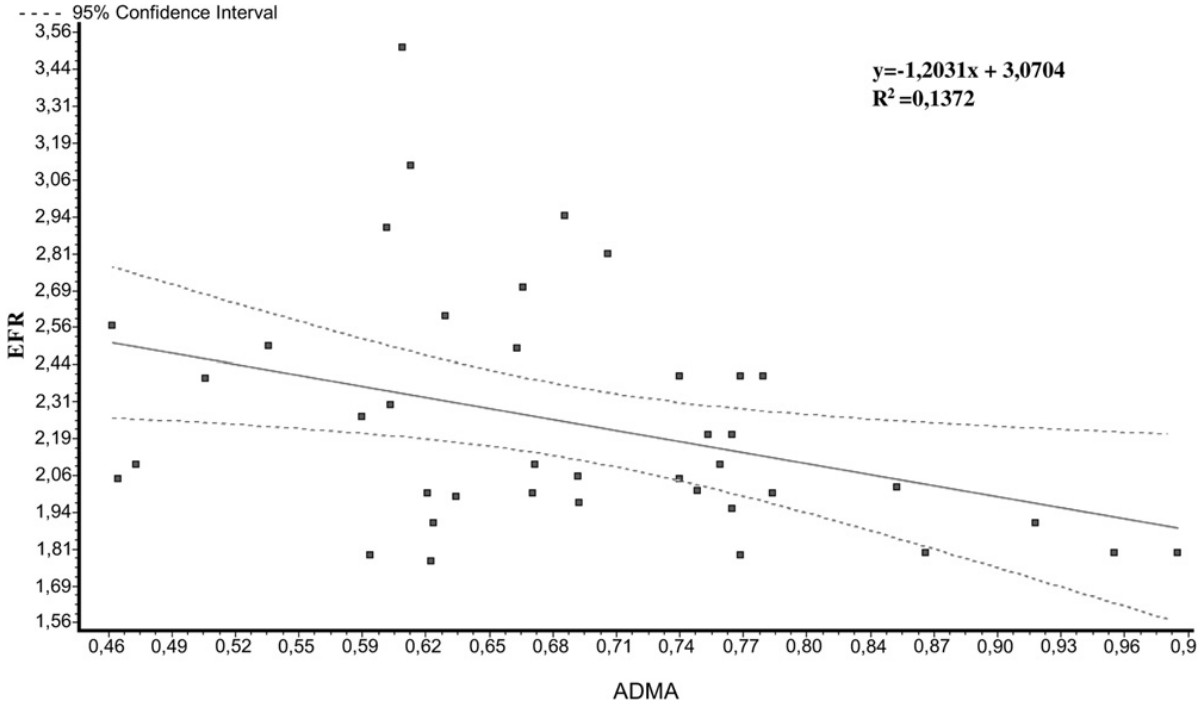


**Figure 2. Correlation between ADMA and EFR**

The significant increase in IL-2 found in AP patients compared with controls confirms a state of chronic inflammation, clearly related to AP.

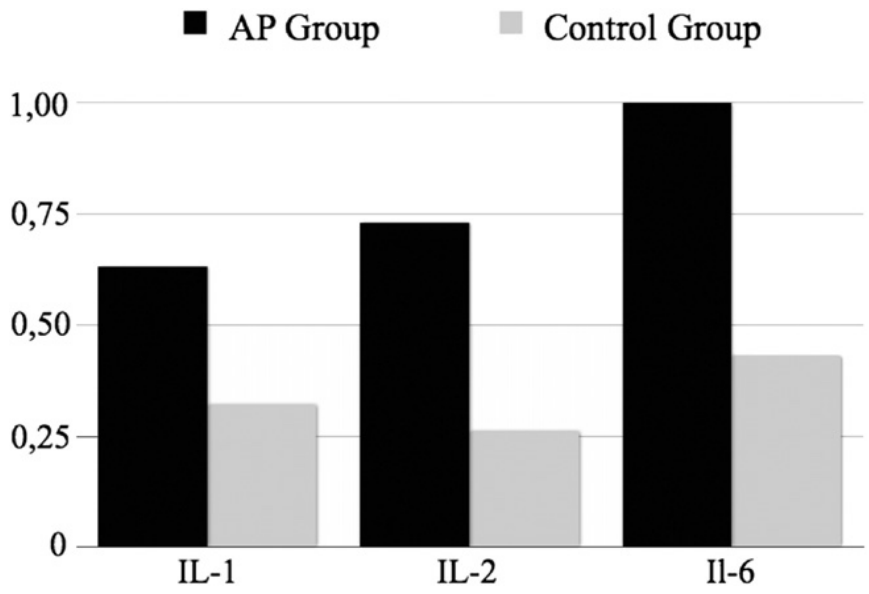


**Figure 3. Levels of inflammation markers in both groups**

Systemic vascular function was evaluated through measurement of ADMA, a naturally occurring component of human blood plasma, which results from the methylation of arginine within the cells. Because of the ability of this substance to inhibit enzyme nitric oxide synthase, elevated ADMA plasma levels are implicated in endothelial dysfunction (Ito A *et al*, 1999; Cooke JP, 2000).

The pathophysiological role of ADMA has been elucidated elsewhere in considerable detail. Major agreement has been reached as to the prominent role played by ADMA in the pathogenesis and progression of CVD, specifically atherosclerosis. An independent relationship between ADMA and the incidence of major clinical conditions such as hypertension (Li J *et al*, 2007), heart failure (Tang WHW *et al*, 2008), coronary heart disease (Zeller M *et al*, 2008) and diabetes (Ellger B *et al*, 2008), and CV death (Anderssohn M *et al*, 2010) has been demonstrated. Serum ADMA levels allow a more detailed risk analysis to be performed when compared with the traditional CV risk markers. Accordingly, the significant increase in serum concentrations of ADMA observed in young AP subjects represents a reliable index of potential endothelial

compromise and should therefore be regarded as a forerunner of future CVD development.

Proinflammatory cytokine-driven responses are fundamental in modulating ADMA. In different clinical and experimental conditions, ADMA levels were markedly higher and correlated with the levels of proinflammatory cytokines (Mookerjee RP *et al*, 2007), supporting the hypothesis that these molecules might regulate ADMA metabolism. In turn, ADMA was recognized as a potential proinflammatory factor (Chen MF *et al*, 2007); exposure of endothelial cells to exogenous ADMA increased reactive oxygen species generation and TNF-a and IL-6 levels (Yang ZC *et al*, 2009). The significant direct correlation between IL-2 and ADMA found in this study suggests a connection between the oral pathosis and the premonitory signs of endothelial dysfunction. Endothelium-mediated vasodilatation in our patients was assessed by means of EFR of digital arteries, a noninvasive parameter of endothelial function. The endothelium is a functional barrier between the wall of the vessel and the bloodstream. It plays a crucial role in the onset of atherosclerotic disease through its vascular regulatory functions, including control of vasomotor tone, local hemostasis, and proliferative processes (Schachinger V *et al*, 2000). Endothelial dysfunction induces thrombosis, vasospasm, and vessel occlusion and has been implicated in the pathogenesis of acute myocardial infarction, stroke, and other CVDs (Muller MM *et al*, 2000).

In the young adult AP patients studied, endothelial function was within the physiological range. However, values obtained were invariably in the lower normal reference range and were significantly lower than in controls. Moreover, EFR values showed an inverse correlation with ADMA, implying how, in the presence of higher plasma ADMA concentrations, EFR values were increasingly compromised. On the basis of the results obtained, it can be assumed that an initial endothelial malfunction is, at this stage, limited to the biological substrate and is therefore inadequate to produce an appreciable hemodynamic insufficiency in young subjects free from traditional CV risk factors. However, the low normal values of EFR and their interdependence with

ADMA levels could foreshadow a possible evolution of endothelial dysfunction in the preclinical vascular lesion or even in an overt CVD.

It is reasonable to hypothesize a cause-effect relationship as underlying the association between AP, IL-2, and high circulating ADMA levels. Thus, AP might represent the primum movens (first cause) of the other effects because of the chronic inflammatory state produced by pathogens inside the root canals of diseased teeth (Nair PNP. In: Cohen S, Hargreaves K, eds. 2006; De Stefano F *et al*, 1993). The release of proinflammatory cytokines into the systemic circulation would appear to be the cause of an increased inflammatory response in distant sites, although not characterized by spread of the infectious agent. The exposure of endothelial cells to cytokines is known to induce a series of chemical-physical changes, including procoagulant activity, up-regulation of chemotactic and adhesion molecules, colonystimulating factor secretion, and monocyte differentiation. These alterations in endothelial cell function, collectively termed endothelial activation, have been implicated in the promoting of acute events in atherosclerotic vascular disease.

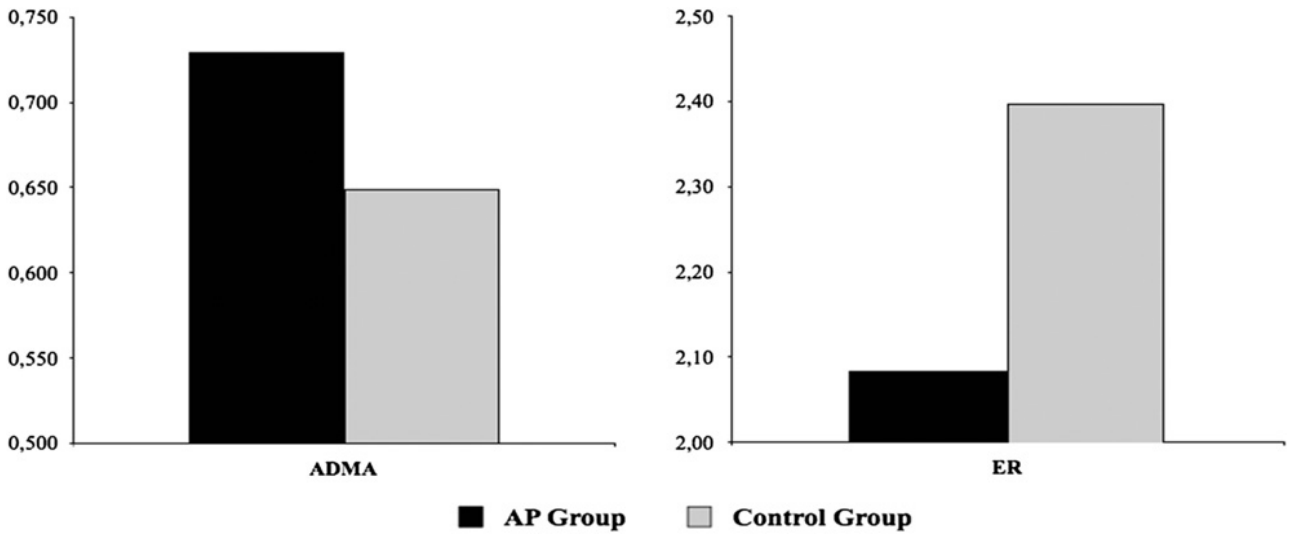


**Figure 4. Levels of ADMA and EFR in both groups**

## ***Developments of the Research***

Further studies will be undertaken to assess the evolution over time of endothelial dysfunction. The study design will moreover be extended with the aim of evaluating the impact of AP in a population at higher cardiovascular risk. For this purpose, the young patients investigated in the present study will be followed until they reach an age acknowledged as involving an increased risk of developing CVD. In consideration of the results obtained, we have also decided that it is most important to assess the levels of ADMA and EFR in the same individuals after endodontic treatment.

Furthermore, a similar study protocol will be set up to investigate a population of women and of older individuals (>40 years) with AP. The findings obtained in this study should further contribute toward determining the reaching of a definitive opinion as to the relationship between endodontic disease and CVD. Indeed, although preliminary, the findings obtained clearly underline the importance of AP as an independent variable in future CVD research and in the development of synergic preventive approaches for both dental and systemic diseases (Hujoel P. 2009).

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Cotti E, Dessì C, Piras A, Flore G, Deidda M, Madeddu C, Zedda A, Longu G, Mercuro G. Association of Endodontic Infection with detection of an Initial Lesion to the cardiovascular System. JOE 2011; 37:1624-9.