

## Chapter 5

# Research Advances in Periodontal Diagnosis

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### Introduction

As our understanding of the etiology, pathogenesis and natural history of periodontal diseases improves, many interesting developments are occurring in periodontal diagnosis.

Periodontal destruction is due not only to the direct effects of pathogenic bacteria, but also to secondary destruction caused by the host response (Genco 1992). Bacteria are necessary, but not sufficient to cause disease, a susceptible host is also required (Loe *et al* 1986). A comprehensive diagnosis should include assessment of systemic risk factors and genetic susceptibility of the diseases, in order to predict risk for further destruction and determine the type and frequency of treatment. Furthermore, periodontal disease does not progress at a constant rate (Socransky *et al* 1984). In actuality it is episodic, with periods of exacerbation and remission. It is important to assess current disease activity, since the type of treatment may vary depending on whether the patient is in an "active" or "quiescent" phase of disease.

As described above, the intent of periodontal diagnosis is not only to provide information about whether the patient is suffering from periodontal disease and which type of periodontal disease is present, but also to

identify current disease activity, predict the risk for future disease progression, guide treatment planning, evaluate treatment outcomes and monitor disease progression during maintenance phase.

Traditional diagnostic procedures, including clinical and radiographic assessments, are still the foundation of periodontal diagnosis, but they have certain limitations. These methods provide only retrospective information about past disease, cannot diagnose disease activity and many are not precise enough to detect small amounts periodontal damage. Hence, many variables may affect the results and as a result the reproducibility of measurements is relatively low. As a consequence of these limitations, it is difficult to monitor disease progression by comparing a series of non-standardized clinical or radiographic measurements.

Therefore, in the past decade much effort has been devoted to improving conventional techniques and developing new diagnostic approaches. This paper reviews important developments in this area and offers a description of traditional and novel diagnostic approaches, including clinical and radiographic techniques, evaluation of microbial challenge, monitoring host biochemical response, genetic susceptibility tests and risk assessment.

## Clinical diagnostic procedures

Traditional clinical evaluation is still the foundation of periodontal diagnosis. Clinicians will typically observe the periodontal tissues for plaque and calculus accumulation and gingival redness, measure probing depth (PD), clinical attachment loss (CAL) and bleeding on probing (BOP). Recently, newly developed electronic devices, including controlled-force electronic probes, Periotest and Periotemp have been introduced to facilitate clinical diagnosis.

The accuracy of periodontal probing can be affected by a number of factors such as the inflammatory status of the tissue (Armitage *et al* 1977), the angle of the probe (Van der Weijden *et al* 1994) and, most importantly, the probing force (Mombelli *et al* 1992). It has been widely recognized that measurements taken with conventional manual probes are subject to a variety of errors. Manual probing cannot reliably measure PD or CAL changes less than 2-3 mm (Hassell *et al* 1973).

Since probing force is the major variable that affects measurement accuracy, in the past decade a variety of controlled-force electronic probes have been invented to minimize measurement errors due to this factor. One of these devices is the Florida Probe. This computer-linked device incorporates constant probing force, automated electronic measurement and direct data entry with computer software. The Florida Probe has been proven to be superior to conventional manual probe as it can reliably measure as small as 1 mm of attachment change (Yang *et al* 1992). This device has not been widely used in clinical practice, partly due to the cost and complexity of the device.

### Other electronic devices

The Periotest is a form of automated

electronic instrument to measure tooth mobility. Periotest delivers a standardized force to a tooth and the time required for the tooth to rebound to its original position is measured on a scale from 0 to 50. This instrument is now commercially available and may be useful for documenting the change of tooth mobility over time.

Subgingival temperature measurement has been suggested as a diagnostic tool for the quantitative assessment of periodontal inflammation. An electronic instrument called Periotemp has been developed for this purpose. It is the shape of a periodontal probe, with a thermocouple device at the end of the handpiece. It allows for collection of temperature, as well as PD and BOP, with one pass of the instrument. Subgingival temperature is measured to a precision of 0.1°C and is then referenced to the sublingual temperature. Sites with higher temperatures had more than twice the risk for future attachment loss than those with lower temperatures (Haffajee *et al* 1992). Further studies are needed to demonstrate the accuracy of this device and its usefulness in clinical diagnosis.

## Radiographic examination

Periapical and bitewing radiographs are invaluable diagnostic aids. They are essential for determining the extent and severity of bone destruction around teeth. However, conventional radiographic assessment of bone levels has four major sources of error:

- 1 Variation in projection geometry.
- 2 Variations in contrast and density due to differences in film processing, kilovoltage and exposure time.
- 3 Masking of osseous changes by other anatomic structures.
- 4 The unaided eye is only able to detect radiographic changes when approximately 30%-50% of bone mineral has been lost.

These factors make it difficult to detect small changes in bone levels using a series of conventional non-standardized radiographs (Hirschmann *et al* 1994).

In the past decade “digital subtraction radiography” has become more widely used in dentistry. It is a computer-assisted image-processing technique used with standardized radiographs. Standardized radiographic films are exposed at certain intervals (every 6-12 months) and digitized. Computer software “subtracts” the initial from the follow-up image, clearly displaying the difference between the two. Bone loss is visualized as a dark area and bone gain as a light area. Thus, the subtraction radiography technique allows the comparison of sequential images over time and helps to identify subtle changes in alveolar bone that would otherwise be missed by unaided eye (Jeffcoat *et al* 1996).

The development of local CT, which distinguishes itself by using a small-field high-resolution detector to generate a limited high-resolution 3D volume, makes it possible to show a patient 3D reconstruction of alveolar bone. This technology is still new, but is very promising for the imaging of alveolar bone.

### **Microbiological tests**

Microbiological tests are not indicated for most adult periodontitis patients, but they may help to more precisely define the cause of disease and guide therapy for specific patients, such as patients with aggressive or refractory periodontitis.

There are a number of advantages in using bacterial markers for periodontal diagnosis. For example, for those specific patients as mentioned above, microbiological testing may provide clinically useful information including the identification of putative periodontal pathogens or unusual superinfecting microorganisms and antibiotic sensitivity, which allows dentists to choose the appropriate

antibiotic chemotherapy. In some cases the bacterial markers appear to be predictive of disease activity. Overall the chairside tests are simple to use and the results are available in a short time. The visual results produced can be shown to the patients and serve as a motivational tool to enhance patient compliance.

Despite these features there are some problems associated with microbiological tests that may limit their diagnostic value. Multiple bacteria are simultaneously involved in the initiation and progression of periodontitis. No single pathogen can be proved to be the cause of disease. Therefore because the levels of putative pathogens necessary to cause periodontal destruction are host-dependent and quite variable, it is difficult to choose certain levels of particular bacterial species to assay as a marker for actively progressing sites of disease. Since it is not possible to sample all the sites in the mouth, dentists must choose ‘appropriate’ sites to sample. The fact that most tests target specific organisms is also a problem. All microbiological tests will only detect the specific bacterial species which are searched for. They are unable to identify ‘unexpected’ or ‘unusual’ species. Finally, the tests are expensive to use on a regular basis.

Because of these limitations, it must be emphasized again that microbiological tests should be reserved for patients with unusual forms of periodontal disease such as aggressive periodontitis or refractory periodontitis. Since these forms usually have a poor response to conventional therapy, microbiological tests may provide useful information to guide treatment plan.

### **Types of microbiological tests**

There are several methods for assessing subgingival plaque samples, including darkfield and phase-contrast microscopy, culture and sensitivity, DNA probes, restriction

endonuclease analysis, polymerase chain reaction (PCR), immunoassay and bacterial enzyme-based assay.

### **Darkfield and phase-contrast microscopy**

This method has been used to detect motile rods and spirochetes (Greenstein *et al* 1985). It has been proven to be of no value in predicting progressing sites in appropriately treated and well-maintained patients.

### **Culture and sensitivity**

Until now, bacterial assays remain the only method that can determine whether bacteria are sensitive or resistant to specific antibiotics. This information can provide important guidelines for antibiotic use. The main drawbacks of this method include:

- 1 Samples must be sent to the laboratory within one or two days to maintain bacteria viability.
- 2 Not all bacteria can be readily cultured.
- 3 The proportional recovery of cultivable species is unlikely to match their proportions in periodontal pocket.
- 4 Only a limited number of laboratories are equipped to grow anaerobic bacteria.
- 5 It takes several days for results to be available.

### **DNA probes**

A DNA probe is composed of a marker molecule and a specific single-stranded nucleic acid sequence that is complementary to the characteristic DNA sequence in specific bacteria (Conrads 2002). Thus, the labeled single-stranded DNA probe can bind to (or hybridize with) the complementary DNA sequence and identify the target bacteria in plaque sample. Bacteria counts are determined by the amount of binding. This method is very

sensitive and can detect as few as 100 bacteria. It is relatively rapid, providing results within 24 hours and it allows concomitant analysis of large numbers of samples with respect to a multitude of bacterial species. Fastidious species not readily grown on culture media can also be identified. Therefore, DNA probes have become a valuable research tool. They are particularly useful in epidemiological studies of microecology, but their usefulness in clinical practice has not been established. This is because probes have only been constructed for a limited number of putative pathogens and they provide no information about the antibiotic sensitivity of bacteria.

### **PCR**

This technology is the most sensitive test currently available for detecting bacteria. A modification of the original PCR technology, “real-time” PCR, permits not only detect of specific microorganisms in plaque, but also its quantification (Conrads 2002). PCR assay in combination with synthesized 16SrRNA probes enable the detection of virtually any microorganisms in dental plaque samples.

### **Immunoassays to detect bacterial antigens**

Each bacterial species has a specific surface antigen that is unique for that microorganism. In immunological assays, a specific antibody labeled with a fluorescent or a colorimetric reaction system is used to bind to and identify the target bacterial antigen in a plaque sample. This technology is very sensitive and at the same time can be very specific if controls are used to check for non-specific reactions (Snyder *et al* 1996). The limitation of the method is that it can only detect species for which a suitable antibody is available.

### **Bacterial enzyme-based assay**

A group of three subgingival pathogens (*Porphyromonas gingivalis*, *Bacteroides forsythus* and *Treponema denticola*) produce trypsin-like enzymes that can degrade a synthetic peptide BANA (Loesche *et al* 1990). Thus, the capacity to hydrolyze this substrate has been incorporated into a chairside diagnostic test called 'Perioscan'. This test employs Evans black dye that produces a permanent blue-black color when reacted with BANA hydrolysis product. The result is read by eye as positive, weak positive, or negative. Positive enzymatic activity indicates the presence of at least one of the three bacteria. This enzyme assay provides a rapid and inexpensive way of screening samples of these bacteria. The main drawback of this test is a lack of quantitative data and the inability to determine which of the three bacteria are responsible for the enzyme production.

### **Biochemical assays of gingival crevicular fluid**

Gingival crevicular fluid (GCF) is a form of inflammatory exudate which can be analyzed to assess local inflammatory process. Biochemical assays of GCF have been extensively studied in an attempt to search for a sensitive biochemical marker to predict disease progression before it can be detected by traditional methods. A very large number of potential markers have been investigated. Some of these have proven to have considerable potential as valuable indicators for disease activity and several commercial chair side kits based on some of these biochemical markers have been developed. However, it is unknown whether they have any diagnostic value in treated and well-maintained patients. It is also unclear whether the results of such tests are applicable to individual sites or if they are best applied to the patient in terms of predictive

value. More longitudinal studies are still needed to confirm the predictive ability of these markers.

In general, these potential markers can be classified into three groups: inflammatory mediator and products, host-derived enzymes and tissue-breakdown products.

### **Inflammatory mediators and products**

#### ***Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)***

This mediator plays an essential role in bone resorption. There is already convincing evidence that correlates PGE<sub>2</sub> levels in GCF to the severity of periodontal disease (Offenbacher *et al* 1986). Sites with periodontitis have significantly increased levels of PGE<sub>2</sub> compared to healthy sites. Level of PGE<sub>2</sub> are markedly elevated during disease progression and appropriate treatment can significantly lower the PGE<sub>2</sub> levels in GCF. This makes PGE<sub>2</sub> a very promising candidate marker for disease progression.

#### ***Cytokines***

Cytokines are potent local mediators of inflammation. Cytokines that have been investigated as potential diagnostic markers for disease activity include IL-1 $\alpha$ , IL-1 $\beta$ , TNF $\alpha$ , IL-6 and IL-8 (Ebersole *et al* 1993), which are associated with bone resorption and neutrophil chemotaxis.

#### ***Antibacterial antibodies***

An important aspect of the host response to periodontal infection is the development of specific antibodies to periodontal pathogens. Studies have shown that there are strong correlations between serum and GCF levels of certain antibodies. However, the clinical usefulness of antibody assays is limited because extensive variation exists on a site and patient

basis with regards to local and systemic antibody production.

### Host-derived enzymes

The inflammation process is associated with accumulation of inflammatory cells and release of various enzymes from these cells. These enzymes can degrade periodontal connective tissue components. Therefore, host-derived enzymes may be good candidate markers for disease progression. Many kinds of enzymes such as neutrophil elastase (Armitage *et al* 1994), neutral proteinases (Eley *et al* 1992),  $\beta$ -glucuronidase (Chung *et al* 1997), MMPs (Ingman *et al* 1996), cathepsins (Chen *et al* 1998), lactate dehydrogenase (Atici *et al* 1998), myeloperoxidase (Yamalick *et al* 2000), tryptase (Eley *et al* 1992), alkaline phosphatase (Binder *et al* 1987), arylsulfatase (Lamster *et al* 1988) and aspartate aminotransferase (Atici *et al* 1998) have been investigated for their association with periodontal inflammation and as markers of disease activity.

### Neutrophil elastase

Neutrophil elastase (NE) is a serine proteinase stored in neutrophil. Studies indicated that GCF samples from sites with periodontitis have significantly higher total NE activity than GCF from healthy or gingivitis sites. Treatment has shown to result in significant decrease in GCF NE level. A commercial chair side test kit has been developed to detect GCF NE. This qualitative test uses filter paper strips impregnated with a synthetic elastase-sensitive oligopeptide substrate. When exposed to elastase, a fluorescent marker is released by the enzyme-substrate reaction that can be detected by ultraviolet light. The intensity of fluorescence is read visually.

### Neutral proteinase

Neutral proteinase is a more general measure of the enzymes that degrade the structural components of gingiva. A chairside qualitative test for neutral proteinase in GCF has been marketed. This test system uses a filter paper strip to collect GCF and then place it on a reaction card impregnated with dye-labeled bovine collagen. As the collagen is lysed, a blue dye appears on the strip. Although the test can detect the presence or absence of the enzyme, the diagnostic value of such information is unclear since the enzyme present at most inflamed sites.

### $\beta$ -glucuronidase

This lysosomal enzyme is found in neutrophils. Elevated GCF  $\beta$ -glucuronidase levels have been reported to have some predictive value in identifying patients who are at higher risk for disease progression. It is unknown if there would be any diagnostic value in a well-maintained population.

### Matrix metalloproteinases

Matrix metalloproteinases are a group of  $Zn^{++}$  and  $Ca^{++}$ -dependent proteolytic enzymes. They are involved in physiological degradation and remodeling of extracellular matrix components. In pathological conditions such as periodontitis, the amount and activity of MMPs will increase markedly, leading to breakdown of connective tissue and periodontal destruction. Recent research has highlighted the importance of MMP-8. It is detected in an active form in the GCF of periodontitis patients, whereas it is mainly in a latent form in gingivitis. It has been reported that more than 80% of periodontal disease activity could be explained by MMP-8, MMP-1 and MMP-2.

### **Cathepsins**

These are a group of acidic lysosomal enzymes. Cathepsins B, H and L are cysteine proteinases that play an important role in intracellular protein degradation. Cathepsin G is a serine proteinase derived from neutrophils. It is secreted with elastase and proteolytically activates latent collagenase. In patients with periodontitis, highly significant correlations have been noted between GCF levels of cathepsins and disease severity. Significant decreases in cathepsins levels have been noted after treatment. It has been suggested that the cathepsins may have some use for monitoring the response to treatment.

### **Aspartate aminotransferase**

Aspartate aminotransferase (AST) is an enzyme normally found in the cytoplasm of cells. Detection of AST in GCF indicates cell death which is believed to be associated with disease progression. Several cross-sectional and longitudinal studies have proved the association between AST and loss of periodontal attachment. A chairside test kit for AST in GCF is currently available.

### **Tissue-breakdown products**

Tissue-breakdown products such as glycosaminoglycans (Embery *et al* 1982), hydroxyproline (Akalin *et al* 1993) and bone specific proteins (Bowers *et al* 1989) have been studied as possible marker for progressive destruction.

### **Glycosaminoglycans**

Glycosaminoglycans (GAGs) are polysaccharide components of proteoglycans, which are widely distributed in connective tissue. Among GAGs, C-4-S has received special attention, since it represents 93.8% of

GAG content of alveolar bone. The appearance of C-4-S in GCF has been suggested as a promising diagnostic marker for bone resorption.

### **Hydroxyproline**

This is a prominent amino acid of collagen and its appearance in GCF has been investigated as a marker for connective tissue destruction. Results indicate that collagen degradation is a prominent feature of both gingivitis and periodontitis, so GCF hydroxyproline level cannot distinguish between sites with gingivitis or periodontitis.

### **Bone specific proteins**

Bone contains a number of proteins within its matrix which are characteristic of mineralized tissue. At sites of periodontitis these bone specific proteins may pass into GCF. Therefore, they have been considered as possible markers of bone resorption and hence periodontal disease activity. These proteins include osteonectin, osteocalcin and telopeptides of type I collagen.

Osteonectin is a noncollagenous protein of bone which is thought to play an important role in the initial phase of mineralization. The amount of GCF osteonectin has been shown to increase in line with the site probing depth, therefore it may be a possible marker for disease severity.

Osteocalcin is a 5.4KD calcium-binding protein and is the most abundant non-collagenous protein of bone. It has been reported that GCF osteocalcin increases remarkably during the development of periodontitis and is significantly correlated with clinical parameters.

Telopeptides of type I collagen (ICTP) is a 12-20KD fragment of bone type I collagen. Elevated ICTP has been shown to coincide with bone resorption rate. In several studies, significantly higher ICTP concentrations in GCF

were found in periodontitis patients. A positive correlation was found between the total amount of GCF ICTP per site and clinical parameters like PD and radiological bone loss.

### Genetic tests

Many preliminary studies have shown that genetic factors strongly influence susceptibility and severity of periodontitis. Periodontitis is a chronic immuno-inflammatory disease. Susceptibility and severity of periodontitis may be influenced by the intensity of host immune and inflammatory response to bacterial LPS. Therefore, genes regulating the production of various immune and inflammatory mediators are potential candidate genes that may influence host susceptibility and severity of periodontitis. These candidate genes include IL-1 gene family (IL-1A, IL-1B and IL-1RN), TNF gene family (TNF and TNF B), COX II gene, Fc $\gamma$ RII A and Fc $\gamma$ RIII B genes. Polymorphisms or mutations in transcription-regulating areas of these genes may lead to inter-individual differences in the production of these proinflammatory cytokines and antibodies. It is proposed that individuals carrying certain genotypes will tend to produce significantly more IL-1 $\alpha$ , TNF $\beta$ , PGE<sub>2</sub>, less IgG<sub>2</sub> or Fc-gamma receptor with lower adherendity to IgG, when challenged by bacterial LPS. These individuals may experience a more vigorous inflammatory reaction or a less effective immune response and be inclined to more extensive tissue destruction.

So far a number of studies have supported this hypothesis. Komman *et al* (1997) reported that a specific IL-1 genotype (namely IL-1A+4845 allele II H/IL-1B+3954 allele II composite genotype) was associated with severe periodontitis. The odds ratio of having this genotype in severe versus mild disease in non-smokers was 6.8. The genotype positive individuals are at higher risk of developing severe periodontitis than genotype negative

individuals (odds ratio 18.9 for age 40-60 years). Recently, a commercial genetic test kit has been introduced to test patient susceptibility for severe chronic periodontitis. It may be concluded that genetic testing has a great potential for future use in disease prevention, treatment planning, making maintenance schedules and preventing over-treatment or under-treatment. A clearer understanding of the genetic heterogeneity of periodontitis may also lead to important revisions to currently used classification system.

### Risk assessment

Risk assessment is a way to identify the potential risk factors for periodontitis so that they may be avoided, reduced or managed. During the past decade many risk factors have been identified. Confirmed risk factors for periodontitis in adults include genetic influences (Rao *et al* 1979), smoking (Haber *et al* 1993), specific pathogens (Haffejee *et al* 1997) and diabetes (Soskolne *et al* 2001). Infrequent dental attendance and poor compliance (Becker *et al* 1984), depression (Aleksėjuniene *et al* 2002) and female osteoporosis (Von Wöwern *et al* 1994) can be considered to be risk indicators of periodontitis.

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