

REVIEW

Traditional versus Microbiological Periodontal Disease Assessment - Where are we today?

Shipra Gupta¹

Associate Professor¹
Department of Periodontia
Dr. Harvansh Singh Judge Institute of
Dental Sciences & Hospital, Panjab University
Sector 25, Chandigarh, India

ABSTRACT:

Most of what we learned about periodontal disease in dental colleges is largely outdated. The old calculus-based theory of periodontal disease has been replaced by a newer paradigm, one derived from microbiological & immunological studies that compel us to rethink the traditional "notched metal stick" approach developed in the 1900's. Presently, the diagnosis & classification of periodontal diseases are almost entirely based on traditional clinical assessments. It has become clear that periodontal diseases are caused by specific, communicable bacteria that trigger a systemic immune response. The immune response does most of the clinical damage, not the microbes. Despite this, most dental professionals still use diagnostic tests developed in the early 1900's such as Disclosing Solutions, Bleeding on Probing, Pocket Depths and Radiographs, all of which are fraught with problems and none of which are predictive of future attachment loss. This article strives to compare the advantages and limitations of traditional clinical assays with the latest microbiological tests currently available.

Key words: Periodontal disease, Risk assessment, Diagnosis, Probing depth.

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INTRODUCTION

It has become clear that periodontal diseases are caused by subgingival microbiota, particularly gram-negative anaerobes.¹ Despite this, most dental professionals still use diagnostic tests developed in the early 1900's such as Disclosing Solutions, Bleeding on Probing, Pocket Depths and Radiographs, all of which are fraught with problems and none of which are predictive of future attachment loss. Questions which come to our mind are; can traditional tests detect pathogenic biofilms? Can they predict future attachment loss? Are more sensitive & specific tests available? Given the fact that periodontal disease are caused by specific bacteria and given the fact that a variety of easy and inexpensive tests are now available, why are we still trying to diagnose

periodontal infections with clumsy surrogate tests from the early 1900's? Isn't it time diagnosis moved beyond ... A Notched Metal Stick? Since we now know that periodontal disease is a transmissible bacterial infection, why not identify the bacteria and treat BEFORE the destruction commences?

DISCUSSION

We Manage What We Measure. If we only measure pockets and bleeding, then that's what gets treated. If we want to treat periodontal infections we need to begin detecting infections during examination. Something's wrong with our present Oral Health System. We are treating "Plaque, Calculus & Pockets", while we should be treating "Wounds" caused by very toxic/virulent organisms. When the gingival attachment is lost due to the pathogenic bacterial invasion, the body has lost an important protective component. As a result, we have an open,

Email for correspondence:
teena1472@yahoo.in

infected wound all the way to bone level. (A truly invasive infection). Why shouldn't we treat this infected wound using the same medical concepts of other infected wound? "Scrapodontics" as the main treatment modality for all types of periodontal diseases is largely outdated. It is essential that we understand that Anti-Infective Periodontal Therapy is a completely new practice paradigm.

It has been proven that 32% of the times, traditional treatment fail within 9 months.² A poor treatment response may be due to persistence of periodonto pathogenic species. It has been reported that a genetic predisposition for inflammatory infections is there.³ However, most patients receive essentially the same anti-infective therapy, despite the recognition that subjects differ in the composition of their subgingival microbiotas. Although all specific pathogens have not been defined, a sufficient number of important pathogens are known and testing for these organisms is, therefore, indicated. Technologies have been developed to the point where rapid microbiological tests are feasible and practical. The rationale for (microbial) assessment and the present level of technology making such assessment feasible strongly supports the use of microbiologic assays as adjuncts in the clinical management of periodontal disease.

Shortcomings of Traditional periodontal tests:

a. Disclosing solution only discloses supragingival plaque which is largely aerobic and non-pathogenic. It cannot differentiate between pathogenic & nonpathogenic biofilms. It is mostly useful for patient motivation.

b. Bleeding on Probing (BOP) doesn't measure disease activity. It measures capillary fragility, which may be the result of disease activity, but more often is the result of iatrogenic factors including medications (Aspirin taken 325 mg daily, for 7 days, leads to an increase of 12.4% in BOP), hormonal levels, operator errors and subconscious expectations. There is no significant correlation between bleeding on probing and other clinical signs and subsequent loss of attachment.⁴ Any force greater than 0.25 N may evoke bleeding in healthy sites with an intact periodontium.⁵ At best it only

indicates disease after the fact and at worse has nothing to do with disease. It is an obsolete "diagnostic" test that refuses to die!

c. Pocket Depth is also of questionable value. At best it is a surrogate measure, not measuring disease but damage after the fact. Like BOP, pocket depth measurements are fraught with inherent errors due to the limitation of trying to make accurate measurements with a notched metal stick. The readings of clinical pocket depth do not normally coincide with the histologic pocket depth because the probe normally penetrates the coronal level of the junctional epithelium.⁶ The pocket is not the disease. It is a result of disease, not the cause. Deep pockets can be risk free & stable. Shallow sites are not protective! Disease originates in shallow sites!

d. Conventional Radiographs are yet another surrogate measurement. They can only detect bone loss *after* the fact.⁷ More than 30% of the bone mass at the alveolar crest must be lost for a change in bone height to be recognized on radiographs.⁸ Radiographs mostly indicate interdental historical damage. They cannot identify pathogenic risk factors. Dentistry, however, is no longer dependent on surrogate clinical tests. A few commercial microbiological tests are now available, including:

1. Direct microscopy - phase- contrast, Dark- field
2. Virulence factors - enzymes (BANA hydrolysis)
3. Specific analyses - Culture and sensitivity
4. Immunologic assays - Immunofluorescence, Enzyme immunoassay
5. DNA/Oligonucleotide probes

1. Direct Microscopy - Evaluation of the subgingival infection can involve assessment of the shapes and motility of the organisms in the subgingival plaque. This can be accomplished with either phase- contrast or, more commonly, dark-field microscopic examination. The dark-field characteristics of the subgingival microflora were systematically evaluated by Listgarten and Hellden.⁹ The presence of spirochetal forms, as well as other organism that are motile under dark-field illumination, has been associated with existing periodontal disease. Although the relationship of dark-field morphotypes to the progression of

Periodontal disease has been investigated in several studies, the results have been inconclusive. Perhaps the presence of spirochetal and motile forms was not a reliable indicator of patients on maintenance who were entering into an active phase of disease.^{10, 11} Other significant disadvantages of dark-field microscopy include the absence of species identification and the lack of guidance concerning the choice of an appropriate antibiotic.¹² Dark-field microscopy is no longer being extensively studied as a diagnostic test for periodontal disease. In brief:

Phase- Contrast Video Microscopy

- a. It can detect a number of high risk bacterial morphotypes including spirochetes and motile cells.
- b. It can also detect WBC's, large numbers of which are the hallmark of periodontal inflammation.
- c. Chairside, 1 minute result
- d. Good for patient motivation, cheap
- e. Can be used for Antibiotic verification (efficacy/compliance)
- f. Disadvantages are: initial cost, unfamiliar technology & no antibiotic specificity, inability to differentiate among the various species of *Treponema*, and inability to identify the main putative periodontal pathogens like *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* as they are nonmotile.

2. Enzyme or virulence factor assays- This type of test depends upon the presence of a virulence factor or other more general markers for microbial activity. The pathologic nature of the subgingival microflora is inferred by the presence of an enzyme or other virulence factors. Studies have focused on the presence of a trypsin-like enzyme in subgingival plaque that can hydrolyze the synthetic substrate benzoyl-DL-argininenaphthylamide (BANA).¹³ The subgingival plaque microorganisms *Treponema denticola*, *Porphyromonas gingivalis*, and *Tannerella forsythia* produce a Trypsin -like enzyme that can hydrolyze BANA. When hydrolysis takes place, it releases the chromophore β - naphthylamide, which turns orange red when a drop of fast garnet is added to the solution. Diagnostic kits have been developed using this reaction (Perioscan). Beck used this test as

a risk indicator for periodontal attachment loss.¹⁴ While this test has been shown to identify periodontally diseased sites with sensitivity and specificity of 80% and 98%, respectively, and a rapid, chairside configuration has been developed, the relationship of this test to active periodontal disease has not been determined. To summarize:

BANA test:

- a. "Red Group" pathogen testing
- b. Chairside, 5 minute results
- c. A highly sensitive and specific enzymatic assay for *P. gingivalis*, *T. forsythus* and *T. denticola*, the three most pathogenic periodontal microorganisms.
- d. The use of the BANA test in clinical periodontal practice has proved to be of high value in aiding the diagnosis of periodontal disease and is of practical applicability in the bacteriologic monitoring of periodontally involved patients.
- e. The test can be compared in card form (Perioscan) and in liquid form. The results are equivalent, with the advantage that the Perioscan method is easier to use and the results can be obtained during the same visit when the plaque sample is collected.
- f. It only detects a very limited number of pathogens, its negative results do not rule out the presence of other important periodontal pathogens.¹⁵ Also, it may be positive at clinically healthy sites.

3. Culture and sensitivity- Gold standard for microbial testing, when determining the performance of new microbial diagnostic methods. Detects 10 disease species. Results obtained in 2 weeks time. The most direct method for identification of the organisms that may be associated with active periodontal disease. It involves growth and identification of micro-organisms from subgingival plaque samples. Clinician can obtain relative and absolute counts of the cultured species. Only in vitro method able to assess for antibiotic susceptibility of the cultured organisms.

Shortcomings: Although non-selective media can be used in such assays, it is recognized that not

all organisms present in the plaque sample will grow equally well, so that the results from the culture may not reflect what is present in situ. Also, some putative pathogens like Treponemas species are fastidious and difficult to grow. Sensitivity of culture methods is low and thus low numbers of a specific pathogen in a pocket are undetected. This test may be modified by the identification of only species previously determined to be important in periodontal disease. This type of test requires viable micro-organisms, a specially equipped anaerobic laboratory, experienced personnel, and is relatively time-consuming and expensive.

4. Immunologic assays- This type of test depends upon the availability of an antibody to specific antigens on the surfaces of target micro-organisms. Either fluorescent or colorimetric detection systems can be used.^{16, 17} By definition, these tests are designed to identify organisms that have been previously determined to be important in periodontal disease. They include:

a. Direct and indirect immunofluorescent assays (IFAs): Direct IFAs employ both monoclonal & polyclonal antibodies conjugated to a fluorescein marker that binds with the bacterial antigen to form a fluorescent immune complex detectable under a microscope. Indirect IFAs employ a secondary fluorescein-conjugated antibody that reacts with the primary antigen-antibody complex. Comparable to bacterial culture in its ability to identify *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in subgingival plaque samples.¹⁶

b. Flow cytometry: involves labeling bacterial cells from a patient plaque sample with both species-specific antibody & a second fluorescein-conjugated antibody. The suspension is then introduced into the flow cytometer, which separates the bacterial cells into an almost single-cell suspension by means of a laminar flow through a narrow tube. Expensive method.

c. Enzyme-linked immunosorbent assay (ELISA): similar in principle to other radioimmunoassay, but instead of the radioisotope, an enzymatically derived color reaction is substituted as the label. Used primarily to detect serum antibodies to periodontal pathogens, can also be

used to quantify specific pathogens in subgingival samples using specific monoclonal antibodies.

Evalusite: a membrane immunoassay adapted for chair-side clinical diagnostic use. It involves linkage between the antigen & a membrane-bound antibody to form an immunocomplex that is later revealed through a colorimetric reaction. Designed to detect *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*.^{17, 18}

d. Latex agglutination: is a simple method based on binding of protein to latex. Latex beads are coated with the species-specific antibody, & when these beads come in contact with the microbial cell surface antigens, cross-linking occurs; clumping is visible in 2 to 5 minutes. Rapid, simple, has great potential for chair-side detection of periodontal pathogens. Not clinically available.

To summarize about immunologic assays:

- i. They provide a quantitative or semi-quantitative estimate of target microorganisms.
- ii. Higher sensitivity & specificity than bacterial culturing
- iii. Do not require stringent sampling & transport methodology
- iv. Not suitable for studying antibiotic susceptibility
- v. The need to develop specific, non crossreacting antibodies is the major problem in the development of these tests. This is a particular concern, since surface antigens on some periodontal micro-organisms (i.e., spirochetes) are poorly defined.

5. Bacterial DNA-PCR Testing - DNA isolated and purified from plaque samples can be analyzed via nucleic acid probes or polymerase chain reaction (PCR).

1. Nucleic acid probes are synthesized sequences of DNA or RNA that are complementary to specific nucleic acid sequences in the bacterial genome. Bacteria can be identified when DNA isolated from dental plaque is hybridized (paired with complementary DNA) with species-specific probes that are labeled to allow visualization. Major advantages of DNA/oligonucleotide probes include the specificity of the reaction, the ability to detect a

relatively small number of the target microorganisms in a sample, and the relative stability of the DNA molecule (for tests that utilize a mail-in sample format for analysis at an off-site laboratory). Probes have been developed for more than 30 periodontal bacteria, and the procedure offers to provide a means of identifying organisms that are difficult to culture (i.e., spirochetes). At present, DNA probe identification of *Actinobacillus actinomycetemcomitans*, *P. gingivalis*, *Treponema denticola* and *Prevotella intermedia* is available (e.g. DMDx, Omnigene). They are more sensitive than culture for *F. nucleatum*, *T. forsythia* and *P. gingivalis*.¹⁹

Checkerboard hybridization is a technique that uses probes to simultaneously test for the presence of up to 43 bacterial species. It enables rapid processing of numerous plaque samples and is often used for research purposes.

2. PCR uses a DNA - replicating enzyme (polymerase) to amplify target sequences of DNA. Standard PCR is not a quantitative assessment of identified bacteria, although a technique called real-time PCR does enable quantification.

Major problems with DNA/oligonucleotide probes include the need for validation of the specificity of the probe for identification of similar species in a genus and the lack of readily available information concerning antibiotic sensitivity of the identified organisms. They require expensive and sophisticated technology. The periodontist must therefore choose one of these two methods according to his specific clinical objective: to obtain rapid, specific detection even with weak initial concentrations (but for targeted periopathogens only) or to be non-specific and analyze the pathological activity with an antibiogram. Hence, on the whole, Nucleic acid techniques should replace cultivation methods as the gold standard in microbiological diagnosis of progressive periodontitis.

Micro-iDent Plus DNA Periodontal Diagnostic Test Kit: This test kit identifies 11 major and minor bacterial pathogens found in periodontal infections; the pathogens are classified into four groups to simplify therapy and antibiotic selection. The simple-to-use test involves inserting a paper point into the

pocket or sulcus and sending it to the lab for testing. Kits are free; each process test costs \$89. Test results are provided electronically with downloadable documents with full color graphs for patient education and archiving in patient files. Use of the test helps identify at-risk patients, determine appropriate therapy, monitor compliance, and create an optimum recall schedule.

Application of diagnostic tests:

- i. Diagnostic tests will find application with patients who have never received periodontal therapy. Baseline data for one or more tests may be part of the information collected when the patient is first examined.
- ii. Following the initial phase of periodontal therapy, there is often a question about the need for additional treatment, and a test that identifies the future, near-term risk for active disease may well be helpful in this situation.
- iii. While the reduction of tissue inflammation and excessive probing depth are fundamental clinical principles in periodontics, diagnostic tests may be used when patients are poor risks for periodontal surgery due to an existing medical condition or age.
- iv. Diagnostic tests are likely to be used for assessment of patients who have received comprehensive periodontal therapy and are on a maintenance schedule.
- v. Identification of therapeutic endpoint that can be used to determine when adequate therapy has been provided.
- vi. The tests can be used to help establish the appropriate re-call interval (i.e., here the test is used to establish a therapeutic endpoint). This type of test would ideally have an *in situ* or in-office format that could provide results before the next appointment is scheduled. The re-call interval could remain the same, could change, or more intensive therapy could be planned.
- vii. Last, a test or battery of tests may be utilized for patients considered refractory to conventional therapy. Since these tests analyze some aspect of the pathologic process (i.e., the presence of a putative pathogen), utilization may help identify why the patient is entering an active phase.

viii. Consecutively, follow-up testing may provide a measure of the effectiveness of therapy.

SUMMARY

Legacy tests are neither precise nor predictive. They are fraught with inherent and operator error. Its time dentistry moved beyond and developed a micro-biologically sensible approach that treats the infection rather than the symptoms & sequel; with far better results.

With an increased understanding of the pathogenesis of periodontal disease, and development of diagnostic tests based on the pathological process, research advances promise to make the treatment of periodontal disease more exciting for the practitioner and more precise for the patient. In general, the usefulness of these tests for predicting future disease activity remains to be established in terms of sensitivity, specificity and predictive value. Although microbiological analysis of subgingival plaque is not necessary to diagnose and treat most patients with periodontitis, it is helpful when treating patients with unusual forms of periodontal disease such as early-onset, refractory and rapidly progressive disease.

Several technical questions must be addressed before these tests can be widely utilized. These specific concerns include such matters as the information available from the tests (e.g. Does the test provide a measure of disease severity or identify the site, region, or patient experiencing active disease?), the most appropriate test configurations, the statistical analysis of data from trials examining the accuracy of the tests, and selection of patients who would benefit from these procedures.

REFERENCES

- 1 Dzink JL, Tanner AC, Haffajee AD, Socransky SS. Gram-negative species associated with active destructive periodontal lesions. *J Clin Periodontol* 1985; **12**: 648- 59.
- 2 Haffajee AD, Cugini MA, Dilbart S, Smith C, Kent RL Jr, Socransky SS. The effect of SRP on the clinical and microbiological parameters of periodontal diseases. *J Clin Periodontol* 1997; **24**: 324-334.
- 3 Kornman KS, Crane A, Wang HY, di Giovine FS, Newman MG, Pirk FW et al. The interleukin- 1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol* 1997; **24**: 72-77.
- 4 Haffajee AD, Socransky SS, Goodson JM. Clinical parameters as predictors of destructive periodontal activity. *J Clin Periodontol* 1983; **10**: 257- 265.
- 5 Lang NP, Nyman S, Senn C, Joss A. Bleeding on probing as it relates to probing pressure and gingival health. *J Clin Periodontol* 1991; **18**: 257-261.
- 6 Polson AM, Caton JG, Yeeaple RN, Zander HA. Histological determination of probe tip penetration into gingival sulcus of humans using an electronic pressure- sensitive probe. *J Clin Periodontol* 1980; **7**: 479- 488.
- 7 Goodson JM, Haffajee AD, Socransky SS. The relationship between attachment level loss and alveolar bone loss. *J Clin Periodontol* 1984; **11**: 348- 359.
- 8 Ortman LF, McHenry K, Hausmann E. Relationship between alveolar bone measured by ¹²⁵I absorptiometry with analysis of standardized radiographs. *J Periodontol* 1982; **53**: 311- 314.
- 9 Listgarten MA, Helldon L. Relative distribution of bacteria at clinically healthy and periodontally diseased sites in humans. *J Clin Periodontol* 1978; **5**: 115-132.
- 10 Listgarten M. Direct microscopy of periodontal pathogens. *Oral Microbiol Immunol* 1986; **1**: 31-38.
- 11 Listgarten MA, Schifter CC, Sullivan P, George C, Rosenberg ES. Failure of a microbial assay to reliably predict disease recurrence in a treated periodontitis population receiving regularly scheduled prophylaxis. *J Clin Periodontol* 1987; **13**: 768-773.
- 12 Listgarten MA. Microbiological testing in the diagnosis of periodontal disease. *J Periodontol* 1992; **63** (Suppl):332-337.
- 13 Loesche WJ. The identification of bacteria associated with periodontal disease and dental caries by enzymatic methods. *Oral Microbiol Immunol* 1986; **1**: 65- 70.
- 14 Beck JD. Issues in assessment of diagnostic tests and risk for periodontal diseases. *Periodontol* 2000 1995; **7**: 100-108.
- 15 Loesche WJ. DNA probe and enzyme analysis in periodontal diagnostics. *J Periodontol* 1992; **63**: 1102- 9.
- 16 Zambon JJ, Bochacki V, Genco RJ. Immunological assays for putative periodontal pathogens. *Oral Microbiol Immunol* 1986; **1**: 39- 44.
- 17 Snyder B, Ryerson CC, Corona H, Grogan EA, Reynolds HS, Contestable PB et al. Analytical performance of an immunologic- based periodontal bacterial test for simultaneous detection and differentiation of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*. *J Periodontol* 1996; **67**: 497- 505.
- 18 Boyer BP, Ryerson CC, Reynolds HS, Zambon JJ, Genco RJ, Snyder B. Colonization by *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* in adult periodontitis patients as detected by the antibody- based Evalusite Test. *J Clin Periodontol* 1996; **23**: 477-484.
- 19 Eick S, Pfister W. Comparison of microbial cultivation and a commercial nucleic acid based method for detection of periodontopathogenic species in subgingival plaque samples. *J Clin Periodontol* 2002; **29**: 638-644.