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Assessing risk and diagnosing in periodontal infection

Periodontal disease is an infection, caused by certain bacteria that stimulate an immune response in the affected individual. When an acute infection becomes chronic, its concomitant sequelae can represent a major public health problem. Chronic periodontal infection has been linked to numerous systemic conditions, including diabetes, cardiovascular and respiratory diseases, chronic kidney disease, preterm and/or low birth weight babies, and even some cancers (1). Studies have been conducted to investigate the relationship between clinical, microbiologic and serologic markers of periodontitis and systemic conditions. This paper will review some assessment and diagnostic tests for risk assessment and diagnosis of periodontal diseases.

Standard protocols for assessment of periodontal infection are clinical periodontal examination, medical history, periodontal probe measurements, clinical attachment loss, radiographic findings, and other clinical features, such as gingival inflammation, the presence and quantity of plaque/calculus, mobility, occlusal problems and other symptoms (2). Supplemental qualitative or quantitative assessments of the gingival crevicular fluid (GCF) and sub-gingival microflora are performed by the collection of dental plaque or saliva, blood samples, to be used in tests to determine oral microbial levels, antigens to oral bacteria, or systemic markers of infection. Supplemental diagnostic tests fall into four basic categories. They can be used to detect the presence of substances associated with putative pathogens; host-derived enzymes; tissue breakdown products; or inflammatory mediators.

Several approaches are used to detect substances associated with accepted periodontopathogens. They include DNA analyses (3, 4), assessment of antigenic profiles (5) and enzymatic activities of certain members of the sub-gingival flora (6, 7). The general intention of all of these assessments is to detect the presence of potentially pathogenic bacteria in sub-gingival plaque samples. They have the advantage of not requiring the collection and preservation of viable bacteria. Most of these tests can reliably identify sites that harbour certain putative pathogens and thus provide information about potential therapeutic targets. *Porphyromonas gingivalis* has been determined as a significant species in the pathogenesis of periodontitis (8–10). *Porphyromonas gingivalis* has also been recognized as having a significant role in systemic disease, such as cardiovascular diseases and atherosclerosis (11–13).

In addition, genetic tests for susceptibility to chronic periodontitis have been introduced into the market. A genetic susceptibility test for severe chronic periodontitis is commercially available that detects the simultaneous presence of a specific form of two interleukin genes, allele 2 at the IL1A+4845 and IL1B+3954 loci. With the PST® test, patients are designated as 'genotype-positive' if both of these alleles are present (14). The foundation of the test is that a combination of these alleles causes increased secretion of IL-β, which results in a hyperinflammatory response to a bacterial challenge, thus increasing risk of a person developing severe chronic periodontitis (14).

A new test slated to be available in 2010 is the GeneEx RPT (Rapid Periodontal Disease Test), which identifies an active periodontal infection, which helps to assess a patient's risk of disease progression and provides evidence of active disease (15). The GeneEx RPT indicates active periodontal infection by detecting an elevated host response to *Actinobacillus actinomycetemcomitans* (A.a) and/or *P. gingivalis* (P.g.) bacteria when they are causing disease. These bacteria, A.a. and P.g., are recognized as the most common and aggressive bacteria causing periodontitis. The test identifies whether a periodontal infection is in progress, allowing the clinician to identify patients most at risk for further disease progression. The presence of an active infection is visually represented and provides immediate feedback on the patient's condition. A positive result indicates the presence of infection caused by the aggressive A.a. and/or P.g. bacteria, a negative result indicates the absence of an existing active infection. The GeneEx RPT predicts active periodontal infections with 92% accuracy (16).

The device is a small, rapid, chair-side disposable lateral flow test where the patient provides a sample of saliva, which is transferred by a pipette to the EDT Cartridge, into an opening at one end of the test cartridge designated as the 'Sample Well'. After 1 min, three drops of buffer solution are added to the EDT Cartridge, and the test result can be read in 10 min. Coloured lines appear in the 'results' window that indicates if the patient has an active infection indicating periodontal

disease. A test line appears, labelled as 'T', indicating a positive test, along with the control line, 'C', indicating the test worked properly. For a negative test, no test line will appear except the control line. This test can be used in dental or medical offices to detect active disease. For more information, go to http://www.geneexinc.com.

The test diagnostics are based on virulence proteins produced by pathogens exclusively during infection and disease and used *in vivo* induced antigen technology (IVIAT). *In vivo* induced antigen technology provides an array of genes that are uniquely active in the pathogenic state, from a marker IVI protein that was selected for each pathogen. These pathogenic state markers are the basis of the RPT, a diagnostic test that identifies the presence of these bacteria in an active pathogenic state.

In vivo induced antigen technology is a technique that identifies pathogen antigens that are immunogenic and expressed *in vivo* during human infection. *In vivo* induced antigen technology is complementary to other techniques that identify genes and their products expressed *in vivo*. Genes and gene pathways identified by IVIAT may play a role in virulence or pathogenesis during human infection, and may be appropriate for inclusion in therapeutic, vaccine or diagnostic applications (17). *Actinobacillus actinomycetemcomitans* is a gram-negative capnophilic rod and the aetiological agent of localized aggressive periodontitis. The genome-wide survey of *A. actinomycetemcomitans* using IVIAT has previously resulted in the discovery of antigenic determinants expressed specifically in diseased patients. One study evaluated the potential of these antigens as assumed disease markers, and investigated their role to the pathogenesis of the microorganism. The data discovered provided new understanding into the pathogenic personality of *A. actinomycetemcomitans in vivo* and supported the use of HeLa cells as a valid *in vitro* host–pathogen interactions model for that microorganism. *In vivo* induced antigen technology is applicable to most pathogens and will undoubtedly lead to the discovery of novel therapies, antibiotics, and diagnostic tools. (*In vivo* induced antigenic determinants of *A. actinomycetemcomitans*.)

The aim of the study was to determine if one of these genes, PG1334, was important for the virulence of *P. gingivalis*. Study of the interaction between *P. gingivalis* and cells from the vasculature, such as human coronary artery endothelial cells (HCAEC), should provide insights into the pathogenic mechanisms of this important bacterium. During an infection, bacterial gene expression constantly changes with disease progression and to adapt to the host environment. (18–20) Previously, IVIAT was used to identify 115 *in vivo* induced genes of *P. gingivalis* strain W83 in human periodontitis (21). The results showed, through analysis of plaque samples from persons with periodontitis, that PG1334 was expressed in 88.0% of diseased sites, compared with 42.1% of healthy sites, even though *P. gingivalis* was detected in equal numbers from both sites. A mutant of PG1334 was found to adhere to and to invade better than the parent strain, but did not persist as well in human coronary artery endothelial cells. Additionally, the mutant did not persist as well in a mouse abscess model. This gene appears to be important for the virulence of *P. gingivalis*, both *in vivo* and *in vitro* (22).

Molecular tests, such as DNA-PCR tests, can assist clinicians in diagnosing periodontal infections and design tailored treatment plans for each patient. This clinical laboratory test is used in medicine to provide important diagnostic information to medical professionals, and is now available for oral healthcare professionals. The DNA-PCR is a polymerase chain reaction (PCR) that uses DNA from bacteria or the DNA of a person to provide information about the pathogenic bacteria that may be found in the oral environment and about the genetic susceptibility traits of your patient. DNA-PCR has utility in two areas: the detection of pathogenic species and the determination of bacterial load of disease causing bacteria; and the determination of genetic susceptibility traits: mutations or polymorphisms (SNP's).

Another test, MyPerioPath test from OralDNA labs, does not require live bacteria (23). This test uses the DNA of dead bacteria to identify the 11 species, (13 bacteria) associated with periodontal disease. This enables the clinician to know the specific type and amounts of pathogenic bacteria that are present. The test can be a part of comprehensive periodontal diagnostic assessment to identify the specifics of active periodontal infections, or used in specific cases. The test samples are gathered in the following manner: using the deepest periodontal pocket in each sextant: Isolate site and do not remove supra- and sub-gingival plaque; With cotton pliers, keep paper point as dry as possible and place to the base of the pocket for 10 s, collecting both supra- and sub-gingival plaque; Remove paper point; place all paper points in collection tube specimen end down; refer to OralDNA – provided Collection Instructions for important details (24) .

As one can see, techniques are becoming more abundant for objectively determining risk for, and the presence of, severe periodontal disease. As mentioned, when an acute infection becomes chronic, its associated sequelae can represent a major public health problem. As periodontitis may add to the systemic inflammatory burden of affected individuals, it is prudent for the clinician to stay informed about new tests and procedures and critically evaluate their utility in clinical practice.

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