3/11 revision

A Description of the BANA Test as it would Relate to Clinical Dentistry.
By Walter Loesche

Overview.

The BANA Test is a simple, inexpensive, chairside in-vitro test which can be used in the dental office. The test is designed to detect the presence of one or more anaerobic bacteria commonly associated with periodontal disease, namely*, Treponema denticola, Porphyromonas gingivalis, and Tannerella forsythia* (formerly called *Bacteroides forsythus)* in plaque samples taken from periodontally diseased teeth or from the tongue coating of individuals with oral malodor. This information may help the clinician in the choice of an antimicrobial agent specific for anaerobes in the case of an individual who presents with advanced periodontal disease of the severity that usually would require surgical intervention. The clinician might also use the presence of these BANA positive species to monitor the adequacy of treatment. The BANA test could alert the clinician to the present of a subclinical/clinical periodontal infection in smokers, or in individuals at risk to cardiovascular diseases or to complications of pregnancy.

Background.

In the early l980's, researchers identified the role of certain gram negative anaerobic bacteria in the emergence and progression of early onset (EOP) and adult periodontitis (AP). (In a somewhat confusing nomenclature decision, the American Academy of Periodontology refers to EOP as aggressive periodontitis (AP) and adult periodontitis as chronic periodontitis (CP)). The most comprehensive of these early studies implicated *Porphyromonas (Bacteroides) gingivalis*, and spirochetes as the species and bacterial types that could be statistically associated with periodontal disease (1). Subsequent studies added the cultivable spirochete, *Treponema denticola* and *Tannerella forsythia* to the list of periodontal pathogens (2-11). Grossi et al. (5,8), in an epidemiological investigation involving over 1,300 adults, identified several risk factors for attachment and alveolar bone loss including the presence of subgingival *P. gingivalis* and *T. forsythia*, as well as smoking. This group did not monitor the third BANA positive species, *T. denticola*, but judging from the literature this species probably would also have been present, as it seems to co-exist with *T. forsythia* and *P. gingivalis* (4,7,10,11). In particular, Socransky and Haffajee in their extensive study involving over 10,000 plaque samples taken from over 100 patients, found the BANA positive species, *T. denticola*, *P. gingivalis*, and *T. forsythia* to have the highest prevalence and to be present in the highest levels compared to over 40 other plaque species that were evaluated by DNA probes (10,11).

Early studies suggested that Aggregatibacter (Actinobacillus) actinomycetemcomitans may also be involved in periodontal disease (2,12,13). But most investigators have failed to find a convincing association between this species and periodontal disease (1,4,5, 8-11). The Grossi et al, and Socransky et al. studies (5,8,10,11), found no evidence that A. actinomycetemcomitans could be associated with periodontal disease. Albandar et al. (14) used DNA probes to assess the relationship between the plaque flora and EOP in 248 US adolescents representative of 14,013 pupils in grades 8 to 12, who were examined in the 1886-87 national survey of the oral health of US children. There was no relationship between A. actinomycetemcomitans and disease progression, but the BANA species, P. gingivalis and T. denticola were significantly associated with loss of attachment.

Thus the preponderance of evidence indicates that *T. denticola*, *P. gingivalis*, and *T. forsythia* are periodontal pathogens and because all are anaerobes, it would appear that periodontal disease is a chronic infection due to the overgrowth of certain anaerobic members of the plaque flora. In vitro these organisms hydrolyze the synthetic trypsin substrate benzoyl DL arginine naphthylamide or BANA (6,7). We were interested as to whether this enzyme could be detected directly in plaque samples and modified the trypsin test so as to be able to use plaque samples. In the initial study, 71% of the plaques removed from untreated periodontal patients were BANA-positive, while only 8% of the plaques removed from successfully treated patients seen at maintenance recall visits were BANA-positive (15). This indicated that the modified trypsin test could be adapted for chair-side use in dentistry, and that it would be of value in the diagnosing of anaerobic periodontal infections. Subsequent development led to the current BANA test which now is a 5 to 15 minute chairside assay (23).

There are over 70 published clinical studies which have used the BANA test. These studies indicate that the BANA test can be used to detect in plaque samples enzymes unique to three anaerobic species, **P. gingivalis, T forsythia** and **T. denticola**; species that have been associated with periodontal disease by DNA probes, by cultural means , and by immunological reagents (2-11). In a recent report, the sensitivity of the BANA test for the identification of **P. gingivalis, T forsythia** and **T. denticola** was 95 %, when compared at the time of the initial diagnosis to a DNA checkerboard procedure (24). It detects these bacteria at levels of about 10^{3-4} cells (7), which is in the range that has reported to be relevant in the causation of adult periodontal disease. (16). While the test does not distinguish which of the three species are present, this distinction may be moot, as the three BANA positive species appear to exist together (7,9-11).

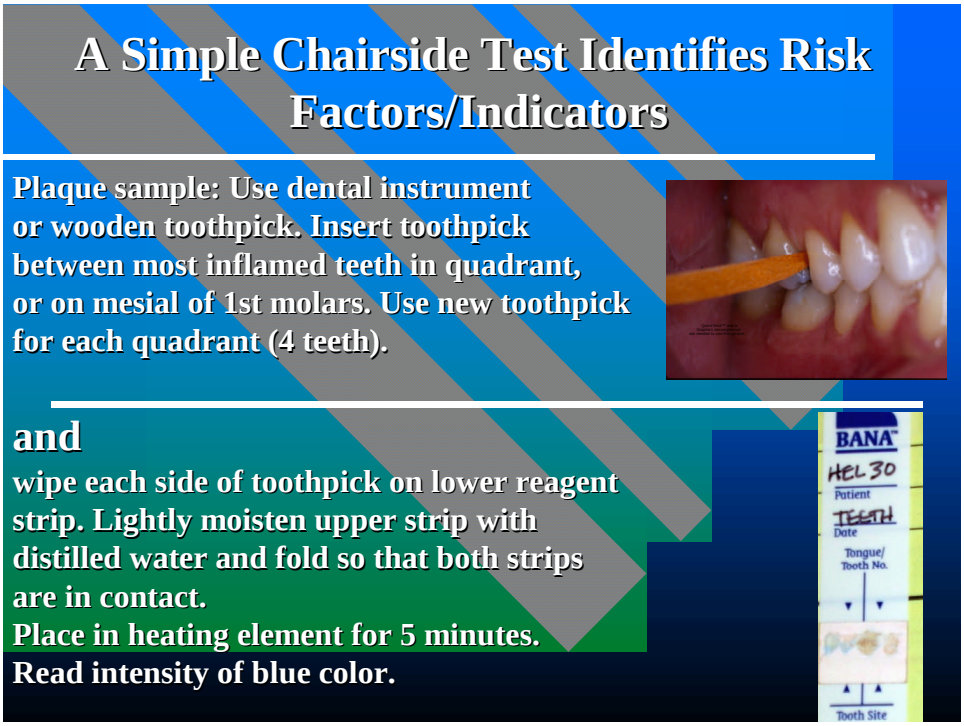
In one of the more important potential utilities of the BANA test, namely the management of an anaerobic periodontal infection, it does not matter which of the three species is present, or if all three are present, because the antimicrobial agent of choice, metronidazole is uniquely active against anaerobes (17,18).

Sampling for the BANA test.

The dentist currently has no inexpensive, in-office procedure by which he/she can determine anything about the character of microorganisms that may be present in plaques removed from periodontally diseased sites. This need is met by the BANA test as it can be used at chairside to give the clinician an immediate feedback as to the presence of enzymes found in **P. gingivalis, T forsythia** and **T. denticola.**

Plaque Samples. Use a dental instrument such as a curette or scaler to remove subgingival plaque and place on lower reagent strip of the BANA test card. We prefer a wooden toothpick such as a STIM-U-Dent® to sample interproximal plaque (Figure 1) because it removes almost a standard size sample, and a new toothpick can be used for each sample, thereby preventing cross-contamination of the samples. Both sides of the flat toothpick are wiped onto the lower strip. The upper strip is lightly moistened with distilled water and the strips are folded together and incubated for 5 minutes at 55 ^{o}C. The strips are removed and the intensity of the blue color is read as weak positive or positive. The detection limit, or weak positive, is about 10,000 cells of **P. gingivalis, T forsythia** and **T. denticola.** A positive test cannot tell which of these organisms are present, or if all are present, but does indicate that one or more of these anaerobic bacteria are present in the plaque.

Figure 1



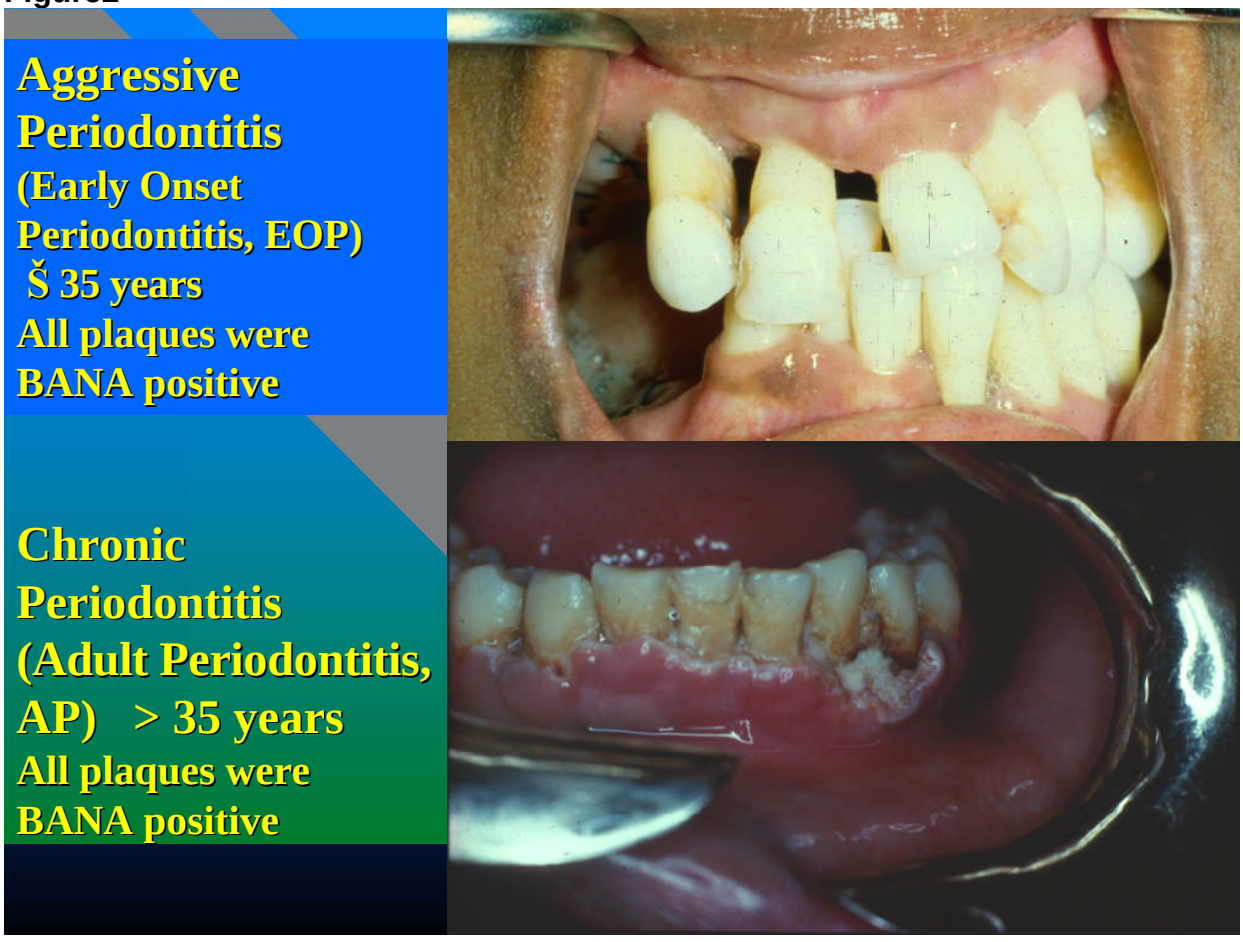
Clinical utilities.

The BANA test could be used to show that clinically diseased sites are colonized by one or more of the three anaerobic organisms commonly associated with periodontal disease. One of the early usages of the BANA test was in epidemiological surveys where Bretz and his colleagues showed that in a field survey of 301 Brazilians, the BANA test had the same sensitivity and specificity as immunological reagents for P gingivalis, T. denticola and T. forsythsis (19). Grisi, Salvador and their colleagues showed that the BANA test correlated well with the CPITN score (20). But the true value of the BANA test is in the clinic, where it can identify patients with an anaerobic infection. In this context, it can be used to diagnose anaerobic periodontal infections and to monitor the adequacy of treatment to reduce or eliminate this infection (17,18).

Diagnosis of an Anaerobic periodontal Infection

Periodontal infections when seen in young individuals under 35 years of age are now called Aggressive Periodontitis to distinguish them from a less aggressive form called Chronic Periodontitis which is found in older individuals (Figure 2).

Figure2



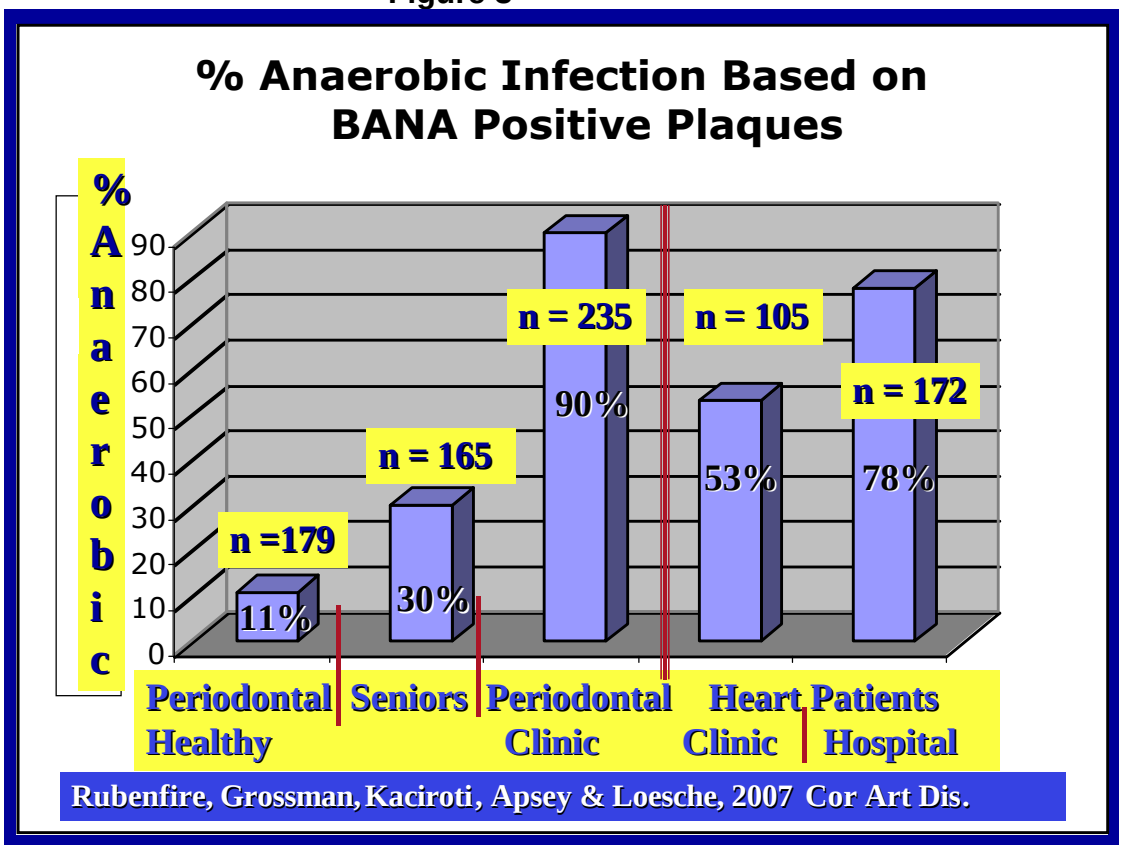
Bacteriologically there is no difference between these two clinical entities in that they both are BANA test positive, have high levels of spirochetes in their plaques, and respond to systemic metronidazole therapy (see below). All four sampled plaques in patients with aggressive or chronic periodontitis were BANA test positive and averaged about 40% spirochetes in the microscopic count. Both clinical conditions are anaerobic infections.

Early Detection of patients at risk to develop periodontal disease

Early detection of sites or patients at-risk to periodontal disease could lead to conservative therapy which may prevent the initiation of periodontal destruction. This would be a more cost effective and humane approach than waiting until after the destruction has begun and can be clinically diagnosed.

We have surveyed a large number of individuals representing different demographic, dental, and medical status for their BANA status. We have sampled the most periodontally involved interproximal site in each quadrant and have diagnosed an anaerobic infection when ≥ 3 or more sites were BANA positive (Fig 3), Eleven percent of 179 dentally healthy individuals ranging from 21 to 64 years, could be diagnosed with a BANA positive infection. The figure rose to 30 % when seniors, i.e., ≥ 65 years, with no dental complaints were sampled. This suggested that an asymptomatic anaerobic periodontic infection was going untreated among these individuals. When patients who had been admitted to a dental school periodontal clinic were sampled, an anaerobic infection could be diagnosed in 90% of the 235 patients. The teeth that were BANA positive were significantly more likely to be recommended in need of periodontal surgery or extraction, than were teeth that were BANA negative (21). We also sampled cardiac patients who were seen in an outpatient clinic after having survived a heart attack, and others who were still in the hospital following treatment for their heart attack. Fifty-three percent of the clinic patients and 78 % of hospital patients had an anaerobic infection (Figure 3).

Figure 3



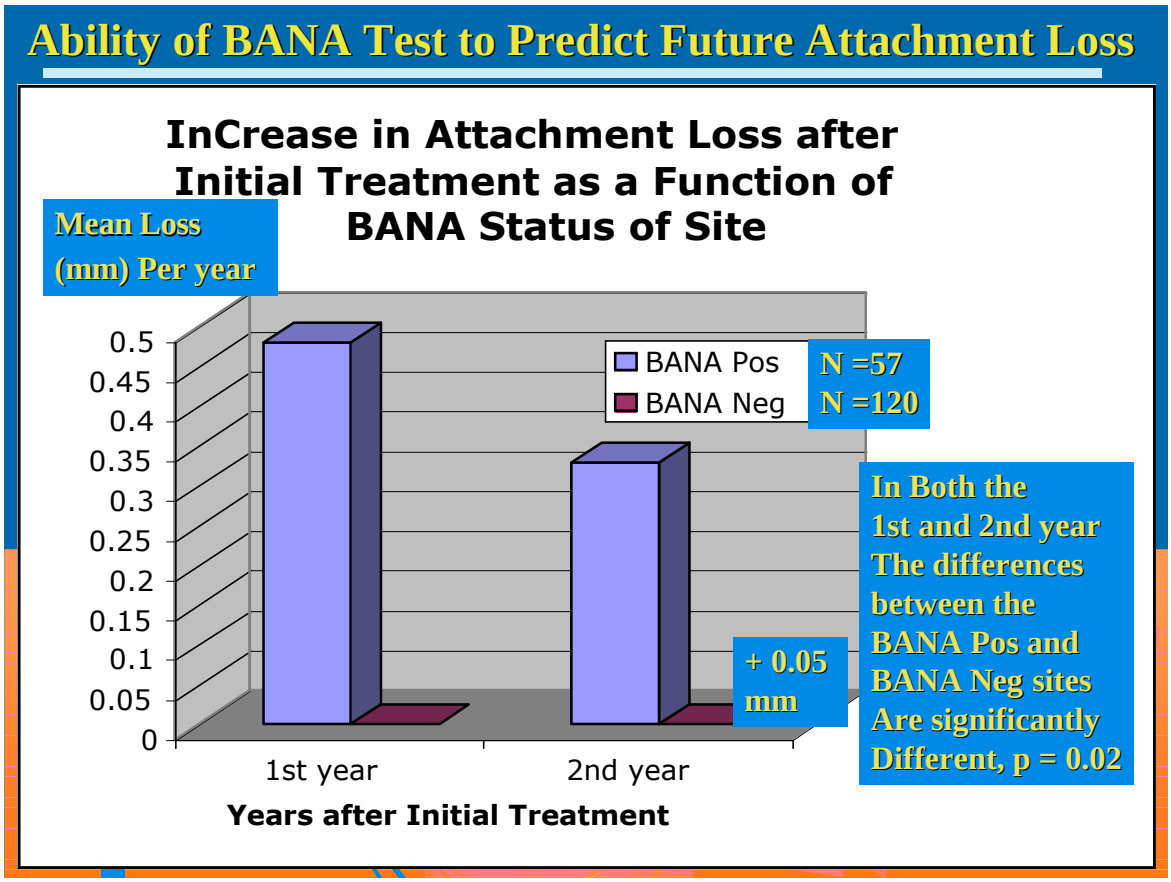
Smokers. The BANA test may be particularly useful in smokers, who are known to be at risk to periodontal disease (5,28,29). Smokers do not exhibit the early telltale signs of a bleeding gingivitis, which could alert the subject or the dentist that he has a possible periodontal problem (30). However, smokers are likely to be colonized with T. forsythia and P. gingivalis (31). We have found that individuals who smoke are almost 10 times more likely to have BANA positive plaques that individuals who do not smoke (23). A positive BANA test in these individuals could alert a clinician of potential (actual) disease and cause him to be more diligent in his treatment of the patient.

Aid in the Management of anaerobic periodontal infections.

After Scaling and Root Planing: The standard treatment in periodontal disease is debridement of the tooth surfaces with dental instruments in a procedure called scaling and root planing. The adequacy of treatment is assessed by the smoothness of the debrided root surface. However, this tactile/visual examination provides no information as to whether the infection has been eradicated. We have tested the tooth surface plaques before and after scaling and root planing and found that many teeth are BANA positive after debridement. One year after treatment the BANA positive teeth had lost almost

0.5 mm of attachment, whereas the BANA negative teeth had not lost any attachment (32)(Figure 4).

Figure 4



Among the teeth followed for two years, the BANA positive teeth had lost 0.3 mm of attachment and the BANA negative teeth 0.05 mm. These differences between the BANA teeth were significant after one and two years. This indicated that the BANA test, performed immediately after debridement, could show that the debridement was, or was not, successful in eliminating the infection. A BANA positive tooth after debridement would suggest the use of antimicrobial agents.

Treatment of Anaerobic Infections: We have interpreted the presence of three out of four, or four out of four BANA positive plaques in patients with overt clinical signs of disease as indicating that the patient has an anaerobic periodontal infection. In a logistic regression model incorporating clinical parameters and the BANA test, the BANA test along with mobility, and level of attachment loss, were significantly associated with the clinician's decision to recommend periodontal surgery as treatment for that particular tooth (21). Patients were entered into a double blind clinical trials of antimicrobial agents based upon:

1). clinical documentation of many deep pockets which would normally require periodontal surgery or extractions, and

2). the presence of an anaerobic periodontal infection as determined by 3 or 4 of the 4 sampled plaques giving a BANA positive reaction.

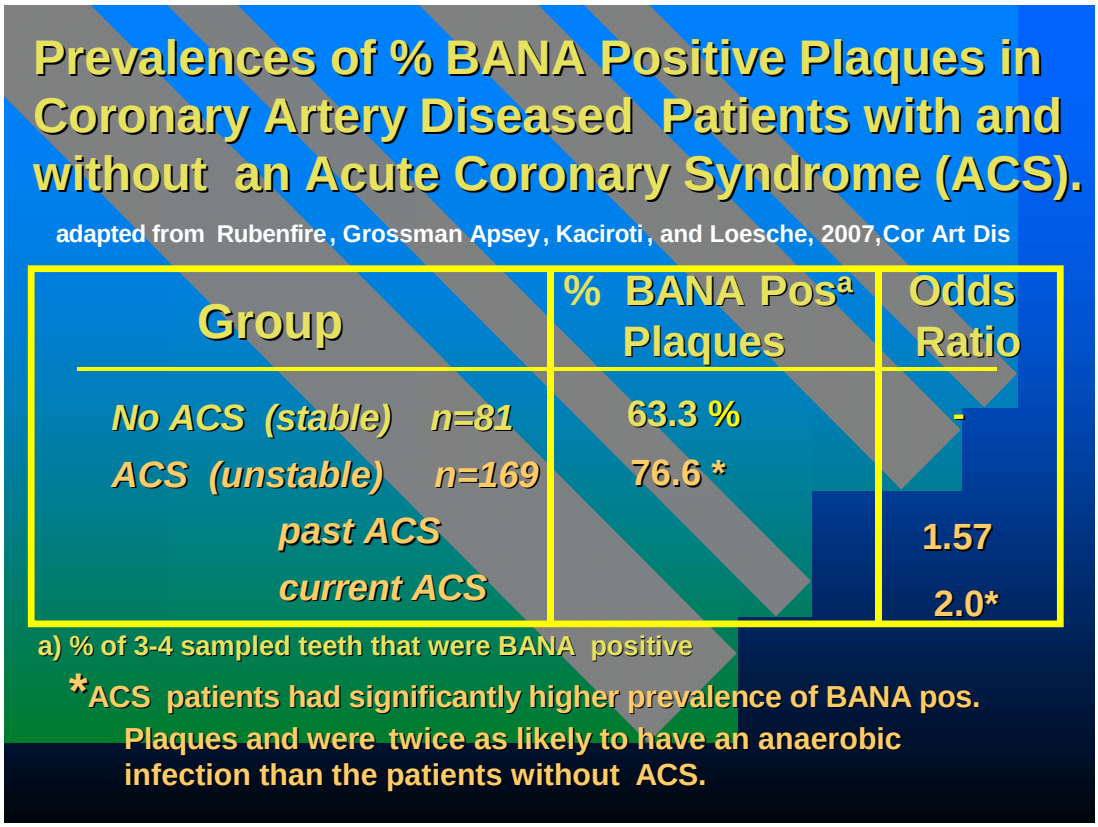
We used as our treatment outcome the reduced need for surgery or extractions. In two separate studies we found that debridement and metronidazole significantly reduced the surgical needs of the patients, when compared to debridement and placebo treatment(17). In a third study we showed that about 90% of the initially recommended periodontal surgical needs could be avoided when patients were treated for two weeks with systemic metronidazole combined with local metronidazole treatment about specific teeth (22). In this last study 96% of the plaques obtained prior to treatment from the 90 patients were BANA positive.

These studies were supported by grants from the National Institute of Dental research so that the cost to the patient was not a consideration. However we estimate that when this approach is transferred to the private sector, this treatment, in addition to being very user friendly, could save the patient, or the insurance provider, from $2,000 to $4,000 compared to the traditional surgical approach. This magnitude of savings means that definitive periodontal treatment will be affordable by many individuals. The choice of metronidazole, which is specific for anaerobic infections, was predicated upon the observation that over 96% of the tested plaques in the individual had a BANA positive reaction.

Screening of Medical Patients for presence of periodontal Infections.

Individuals with Cardiovascular Disease. In a recent development investigators have shown an association between dental disease and cardiovascular disease (33-39). We have found that in older patients that the BANA test was a significant independent risk indicator of coronary heart disease (CHD), using statistical models in which cholesterol levels, and body mass index were not significant (40). Patients with a diagnosis of CHD were 2.06 times more likely to have all 4 plaque samples testing BANA positive compared to subjects who did not have CHD. Within the cardiac patients is a group with unstable angina that are diagnosed with an Acute Coronary Syndrome (ACS). The ACS patients had a significant higher prevalence of BANA positive plaques and were twice as likely to have an anaerobic periodontal infection compared to patients without ACS (Table 1). Thus within the cardiac patients the BANA test might be able to diagnose those patients at greater risk of a recurrent heart attack.

Table 1



This presence of BANA positive plaques would indicate that periodontopathic species were elevated on the tooth surfaces of subjects with CHD. These BANA-positive species are gram-negative anaerobes, so that their elevation in the dental plaque would support the various hypotheses linking chronic bacterial infection to CHD via effects mediated by endotoxin or lipopolysaccharides [41-43].

In a study involving 657 dentate subjects, the levels of bacteria on the teeth, and in particular, P. gingivalis, T. denticola and T. forsythia, as measured by DNA probes, were shown to be independently related to carotid artery intima-media thickness. This represents direct evidence for a relationship between periodontal microbiology and subclinical atherosclerosis (44). Other investigators have shown that periodontal disease, defined as the percentage of periodontal sites with pocket depth of 4 mm or more, as well as BMI were jointly associated with increased C-reactive protein (CRP) levels in otherwise healthy, middle-aged adults (45). In a study involving older individuals, BANA positive plaques were associated with higher plasma levels of CRP after controlling for established risk factors for increased levels of these markers (46). These findings suggest the need for medical and dental diagnosis when evaluating the sources of acute-phase response in some patients.

The role of the BANA test in identifying individuals at risk for periodontal disease and possibly CHD is speculative at this time, but its potential importance is great. If periodontal disease is a risk factor for cardiovascular disease it would

be a **modifiable risk factor**, that could be reduced by the early treatment of periodontal conditions. Such a possibility has been reported from Austria, where investigators tested endothelial function in 30 patients with periodontal disease and 31 control patients (47). The patients were treated by scaling and root planing, the use of chlorhexidine mouthrinses for 14 days and systemic antimicrobials for 7 days. The results showed that periodontal treatment reversed endothelia dysfunction, but further studies are needed to determine whether this will translate into a beneficial effect on atherogenesis and subsequent cardiovascular events.

Detection of women who are at risk of delivering a pre-term birth.

The prevalence of preterm births (PB) is often associated with infections and/or inflammations, including periodontal infections(48). A field study in Taiwan involving 268 pregnant women showed that BANA-positive plaques in the third trimester were associated with preterm births after controlling for other risk factors (49). This suggests that the BANA test can be used to screen pregnant women at chairside and/or bedside to identify women who might benefit from periodontal treatment. When periodontal treatment is successful in pregnant women there is a significant reduction in pre-term births (50a).

Placement of Implants.

The placement of dental implants has been one of the most successful innovations in clinical dentistry. Some implants fail because they are placed in a mouth infected with anaerobic periodontal species which results in an infection about the implant known as peri-implantitis (50-53). Implants that develop peri-implantitis usually exhibit an infection due to the overgrowth of the BANA positive bacterial species associated with periodontal infections. In one study (54a), 79% of the peri-implantitis teeth had a BANA positive reactions which was significantly greater than the 17% positive BANA reactions about healthy implants in

asymptomatic patients.

In order to minimize the occurrence of peri-implantitis the clinician could determine the BANA status of the teeth about the implant site(s), and if positive, treat with local or systemic antimicrobial agents until the infected teeth become BANA negative. Once these teeth become BANA negative, the likelihood of a subsequent peri-implantitis would be reduced. This strategy is illustrated in Figure 5 which shows the implant being placed in a patient whose teeth, adjacent to the implant site, are BANA negative. This prevention strategy should greatly reduce the likelihood of subsequent peri-implantitis.

Figure 5

Individuals with Oral Malodor

Many individuals complain of oral malodor, and often seek treatment for such. About 90% of oral malodor originates from the tongue and reflects the proteolytic activity of oral anaerobes. These bacteria degrade peptides, and proteins releasing volatile sulfur compounds (VSCs), volatile fatty acids and other compounds such as putrescene which contribute to the oral malodor (54). The volatile sulfur compounds can be detected with a sulfide monitor called the Halimeter®, but there is no way to detect the other malodorous compounds. The BANA bacteria in vitro produce a variety of foul smelling compounds such as VSCs, and fatty acids such as valeric, propionic, butyric and others. We and others (55,56) have found that small samples of tongue coatings are BANA positive in individuals with oral malodor. In fact a positive BANA test correlates with all the oral malodor parameters and when combined with the Halimeter® readings improves the correlation of the combined readings with organoleptic scores (56). When patients are successfully treated to reduce and/or eliminate the oral malodor the tongue BANA scores convert from positive to negative (Kazor and Loesche, unpublished data). This finding suggests that the BANA test could be used to monitor treatment efficacy in reducing malodor.

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