The significance of BANA test in diagnosis of certain forms of periodontal disease

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Summary

Many paraclinical methods are available today for an accurate assessment of the periodontal status prior and during the periodontal treatment. The microbial-enzymatic BANA test is one of the modern alternatives to bacterial cultures. It detects the presence of three periodontal pathogens in the subgingival plaque (*T. denticola, P. gingivalis, and B. forsythus*).
The *objective* of the study is to present the clinical importance of using BANA test for the paraclinical examination of patients with periodontal disease and also to show if there is a statistical correlation between the severity of periodontal disease and the results of the test.
***Method*. This study included 61 adult patients, all exhibiting gingivitis or periodontitis. Periodontal charts and BANA test were performed in all patients.**
The *results* show that the BANA tests are statistically correlated in our study with the severity of periodontal destruction. There was no statistical correlation between the BANA test results and the quantity of bacterial plaque, the test being influenced by the composition of bacterial plaque.
The *conclusion* of the study encourages the use of such chair-side tests for a proper diagnosis of periodontal disease and for a good evaluation of the treatment results.

***Keywords*: gingivitis, periodontits, BANA test.**

Introduction

The microbial etiology of periodontal disease is well known. The pathogenic bacteria are growing in the oral biofilm, the gram negative anaerobic species developing in subgingival area being more aggressive for the periodontal structures [1].

In the early 1980's, researchers identified the role of certain gram-negative anaerobic bacteria in the emergence and progression of adult periodontitis. The most comprehensive of these early studies implicated *Porphyromonas gingivalis* and spirochetes as the species that could be statistically associated with periodontal disease [2]. Other studies [3] implicated the cultivable spirochete, *Treponema denticola* and *Bacteroides forsythus* as also being involved in the periodontal disease. Grossi et al. [4,5], in an epidemiological investigation identified several risk factors for attachment and alveolar bone loss, including the presence of subgingival *P. gingivalis* and *B. forsythus*, as

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well as smoking. Socransky and Haffajee (1987) 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 *B. forsythus* 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 [6,7].

Currently, there are many methods directed at identifying periodontal pathogenic species: microbial cultures, DNA probes, polymerase chain reaction. The latest two methods can detect uncultivable species, but they require good laboratory equipment and they cannot be used as routine tests [8].

Another category of tests includes the chair-side tests, biochemical and enzymatic tests. BANA test (*Figure 1*) is a modern chair-side paraclinical method designed to detect the presence of one or more anaerobic bacteria commonly associated with periodontal disease, namely *Treponema denticola, Porphyromonas gingivalis and Bacteroides forsythus* in subgingival plaque samples taken from periodontally diseased teeth.

The BANA test (*Figure 1*) was developed by Dr. Walter Löesche and coworkers at Michigan University, being the result of more than 15 years of research.

Of the 60 bacterial species studied in the subgingival microbiota, only the anaerobic bacteria *Porphyromonas gingivalis, Bacteroides forsythus* and *Treponema denticola* possess a trypsin-like enzyme, which hydrolyzes the synthetic peptide benzoyl-DL-arginine-naphthylamide or BANA. The test can detect the presence of these three anaerobic species, without being able to differentiate them [9].

The BANA test is very sensitive, detecting small quantities of pathogens. No meaningful differences could be found between DNA probes, immunological reagents and the BANA test, when seeking to detect these species in plaque samples removed from periodontal disease patients [10,11,12].

The test can be used for assessment of oral halitosis, to detect the presence of two BANA positive species on the tongue surface: *Stomatococcus mucinlagenous* and *Rothia dentocariosa.*

Figure 1. BANA test

Material and method

Principle of BANA test

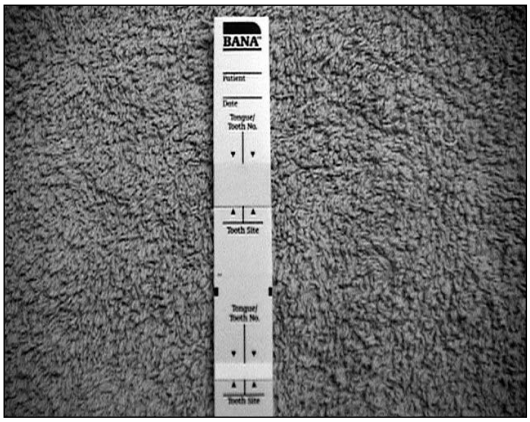
Peptidases of these three bacterial species (*T. denticola*, *P. gingivalis*, and *B. forsythus*) can hydrolyze the peptide analog N-benzoyl-DL-arginine-2 naphthylamide (BANA). One of the hydrolytic products of this reaction is B-naphthylamide, which reacts with a reagent, which is imbedded in the upper strip of the test, producing a permanent blue color. Blood and saliva do not interfere with the test [13].

The BANA test is a plastic strip to which two separate reagent matrices are attached (*Figure 2*).

- The lower white reagent matrix is impregnated with N-benzoyl-DL-arginine-B-napthylamide (BANA). Subgingival plaque samples are applied to this lower matrix.
- The upper buff reagent matrix contains a chromogenic diazo reagent, which reacts with one of the hydrolytic products of the enzyme reaction, form-

ing a blue color. The blue color appears in the upper buff matrix and is permanent. The intensity of the color determines whether it is a positive or weak reaction.

Figure 2. BANA test strip with the lower matrix for the plaque samples and the upper matrix with the reagent



Directions for use

Anaerobic microorganisms associated with periodontal disease are found in the subgingival plaque. To obtain specimens for testing, sites should be cleared of supragingival plaque. A Gracey curette may be used to obtain subgingival plaque specimens (*Figure 3*), which are placed on the lower matrix. Four teeth should be sampled in each subject. Before taking another specimen, wipe the curette on a clean piece of cotton or other suitable wipe to prevent carry-over of plaque.

Then the upper matrix is moistened with saline solution and the test is folded so as the two matrices are coming in contact. It is incubated for 5 minutes at 55 Celsius degrees temperature.

If BANA positive species are present when the test is opened, a permanent blue coloration on the upper matrix is found (*Figure 4*). The higher the concentration of bacterial species, the darker blue coloration is present on the test. According to the result, the test can be positive, weak positive, or negative.

Figure 3. Gracey curette is used to obtain subgingival plaque samples in a patient with adult periodontitis (patient included in the study)

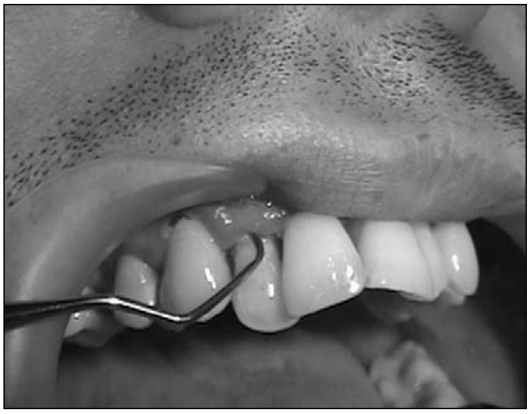
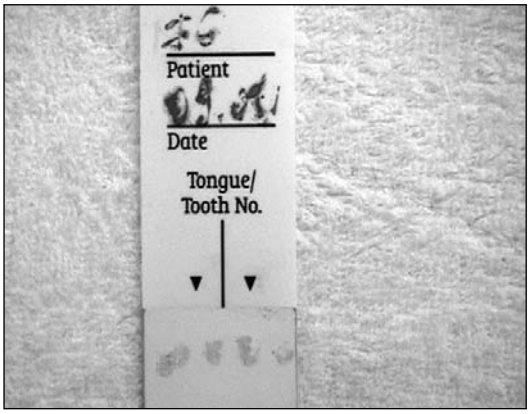


Figure 4. The positive BANA result in all four samples



Study protocol concerning the importance of BANA test in periodontal diagnosis

The study was developed between 2003-2005 on a group of 61 young patients, with ages between 15 and 46 years old, all systemically healthy. All patients included in the study had a certain form of periodontal disease.

The purpose of the study is to show the importance of microbial-enzymatic BANA test in the examination and treatment planning of patients suffering from periodontal disease and also to establish if there are statistical correlations between BANA results and the severity of periodontal destruction.

Patients that fulfilled the study protocol

agree that their data are included in the statistical study.

All patients were examined and periodontal charts were recorded, according to the model used in Constanþa and Bucharest chart of Periodontology disciplines, devised by Professor H.T. Dumitriu. To assess the degree of oral hygiene, the debris index (DI) was used, which results from plaque index (PI) and calculus index (CI) [14].

MedCalc®, version 7.3.0.1. was used for the correlation analysis between BANA results and severity of periodontal disease.

To ease the statistical evaluation, all the variables were given numerical correspondents, as follows:

The clinical forms of periodontal disease were marked with the following numbers:

1 - for the plaque-induced gingivitis;

2 - for the chronic marginal superficial periodontitis;

3 - for the adult periodontitis.

Similarly, the BANA test results were coded as follows:

1 - for the negative BANA result;

2 - for the weak positive BANA result;

3 - for the positive BANA result.

Spearman's rank correlation coefficient was used for calculation of the statistical determinant.

The result is a positive statistical correlation between the clinical form of periodontal disease (PD) and the BANA test result, proved by an **rho** correlation index of 0.752, which is a value close to 1, and a ***P*** value which goes to zero (*Table 1*). This positive correlation existed in a previous study [15].

Table 1. Correlation between the BANA test results, clinical form and PI (Spearman Coefficient)

	PD		**PI**	
	rho	P	rho	P
BANA	0.752	<0.00001	0.325	0.0118

On the contrary, there is no relevant statistical correlation between the BANA test results and the level of oral hygiene evaluated with plaque index (PI). In this relation, the **rho** correlation index has a small value, close to 0 than to 1 and the P value is quite big (P reflects the probability of obtaining our result if the null hypothesis is true) [16].

Results and discussion

Comparing the results of BANA test with the corresponding clinical forms of periodontal disease we noticed the following:

In patients with plaque-induced gingivitis, BANA test was negative in most cases, which suggests the absence of the three pathogenic species, or if present, in reduced numbers (less than 10,000 colony-forming units) in each plaque sample. There are few cases of patients with plaque-induced gingivitis with a longer evolution, which give a BANA weak positive result. This result suggests the risk of disease evolution to periodontitis.

Most patients with chronic marginal superficial periodontitis had BANA weak positive or negative BANA results. A weak positive result in a patient with existing signs of inflammation and demineralization affecting the alveolar bone shows an imminent risk of developing loss of periodontal structures with the occurence of adult periodontitis. This finding is correlated with the evolution possibility of chronic marginal periodontitis, which untreated will always lead to the irreversible destruction of periodontal structures.

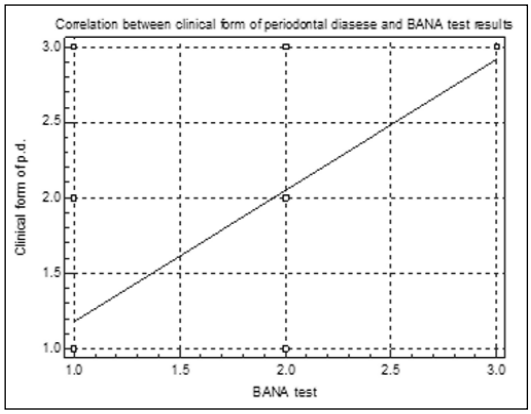
The majority of patients with adult periodontitis recorded BANA positive or weak positive results, which means that this clinical form is accompanied by the presence of BANA positive bacterial species (*Treponema denticola, Porphyromonas gingivalis* and *Bacteroides forsythus*) in subgingival plaque.

A BANA positive result in patients with

objective signs of periodontal inflammation indicates the presence of more than 10,000 colony-forming units of bacterial species in each plaque sample [10,11]. These patients need to follow systemic and local antimicrobial therapy in order to reduce bacterial colonization of subgingival areas.

Chart 1 shows a theoretical graphic representation of the relationship between the clinical form of periodontal disease and the BANA results

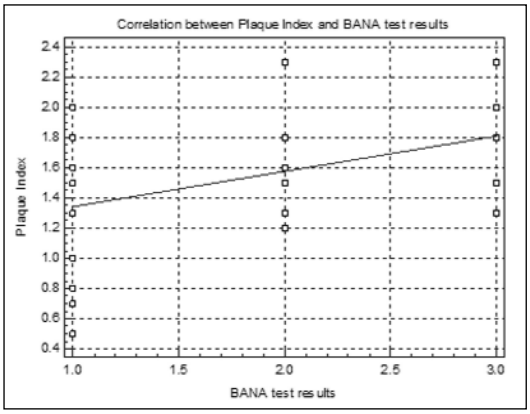
Chart 1 - Made with MedCalc®



Comparing the values of plaque index PI and the results of BANA test we observe that there is no good statistical correlation between these two variables. **Rho** correlation index is small as value, 0.325 and ***P*** value is 0.0118 (*Table 1*). The reason why a good correlation between these two variables does not exist is the different composition of bacterial plaque. So, the PI includes both supragingival and subgingival plaque deposits, the first ones being poor in pathogenic anaerobic species, BANA positive. The BANA test was done using only subgingival plaque deposits, with a different microbial composition.

The theoretical graphic representation of the relationship between PI and BANA test results is seen in *Chart 2*. For a BANA negative result most of the PI values are varying between 0.5 and 2 and for a BANA positive result the PI values are higher, between 1.3 and 2.3.

Chart 2 - Made with MedCalc®



Conclusion

There is a positive correlation, both clinically and statistically, between the BANA test results and the current stage of periodontal destruction.

The BANA test results are not correlated with the degree of oral hygiene evaluated against the plaque index, so the quality and not quantity of bacterial plaque influence the test results.

For the detection of periodontopathogens, microscopy, culture, immunoassays, enzyme tests, and DNA-based techniques have been introduced [17]. Among these possibilities, the microbial-enzymatic BANA test is a quick, chair-side test with a very good sensibility, giving the clinician details about the microbial composition of the subgingival plaque and consequently about the clinical evolution of the periodontal disease. BANA test is also offering therapeutic orientation regarding the need for antimicrobial therapy.

This microbial-enzymatic test gives the dentist a realistic image of the degree of bacterial accumulation of BANA positive pathogens, reason why this test can be used in large epidemiologic studies concerning the severity of periodontal infection with germs, which have a potential risk in the occurrence and evolution of heart disease and premature birth.

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