Biochemical and enzymatic diagnosis aids in periodontal disease

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Summary

Among the paraclinical methods that can be used for an accurate assessment of the periodontal status prior and during the periodontal treatment we present the importance of using two tests, the biochemical TOPAS test and the enzymatic BANA test. Both tests have a good practical importance proven by statistical good correlation between the results of TOPAS and the papilla bleeding index, and BANA tests and the clinical form of periodontal disease.
The conclusion of the study encourages the use of such chair-side tests for a proper diagnostic of periodontal disease and for a good evaluation of the treatment results.

Keywords: gingivitis, topas test, periodontits, bana test.

Introduction

It is known that periodontal disease is produced by the bacteria residing in the oral biofilm, named also bacterial plaque. It is difficult to isolate and grow many of those bacterial species by the culture method because this requests good equipment and even though, some of the bacterial species are uncultivable [1]. The DNA probes and polymerase chain reaction (PCR) techniques can detect even the uncultivable bacteria [2,3], but they require good laboratory equipment and therefore are not used as routine examination in the dental surgery.

In 1992, dr. L□esche introduced a chair-side quick microbial-enzymatic test, BANA, in order to assess the presence of some bacterial pathogen species in the subgingival plaque [4].

Recent studies have shown that the progression of periodontal destruction due to bacterial pathogen species is episodic and does not occur in a continuous, linear manner. Furthermore, periodontal pockets can be active or inactive in the disease process [5]. Conventional diagnosis indicators, such as probing depth, attachment level, bleeding on probing, gingival redness, gingival index, and assessment of alveolar bone on radiographs, cannot distinguish between disease activity and inactivity. Additional diagnosis information is therefore needed.

The analysis of crevicular fluid [6] is one possibility, because its components show the etiopathogenical phenomena produced in the periodontium, being considered as markers of the progression and severity of periodontal disease. This can be done by a biochemical test named TOPAS (TOxicity Prescreening ASsay), which detects the indirect presence of bacteria by two markers of gingival infection: bacterial toxins and bacterial proteins. This test can be associated with the severity of inflammation and with the evolution of a destructive process, making the difference between an active and an inactive periodontal disease. We have described the practical importance of TOPAS test in a previous work. This work

follows the previous research and its aim is to check if there exists a statistical positive correlation of those tests with clinical findings and with the severity of periodontal involvement.

Materials and Method

1. The principle of BANA test is to detect the presence of three anaerobic bacteria associated with periodontal disease by the analysis of subgingival plaque. These three bacteria are: ***Porphyromonas gingivalis, Treponema denticola,*** and ***Bacteroides forsythus.*** Socransky and Haffajee (1997) concluded that these three pathogens have a great prevalence in patients with periodontitis [7]. BANA test can detect the *presence* of these three microorganisms associated with periodontal disease but it cannot make the difference between them [8]. The BANA positive bacterial species contain a certain enzyme that can hydrolyze the peptide impregnated on BANA strips. This peptide is N-benzoyl-DL-arginine-B-napthylamide (BANA). When samples containing any of the three bacteria are placed on a BANA-impregnated test strip, a hydrolytic reaction gives the strip a distinctive blue color. The darker the blue, the more organisms are present.

The test (*Figure 1*) consists of a plastic strip to which two separate reagent matrices are attached. The lower white reagent matrix is impregnated with N-benzoyl-DL-arginine-B-napthylamide (BANA). Subgingival plaque samples are applied to the lower matrix. The upper buff reagent matrix contains a chromogenic reagent that reacts with one of the hydrolytic products of the enzyme reaction to form a blue color. The upper matrix should be moistened with saline solution after sampling. Then the plastic strip is folded (so the two matrices come in contact) and placed for 5 minutes in incubator, which is set at 55 °C. 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 positive reaction.

Figure 1. The BANA recipient containing 20 test strips

Figure 2. TOPAS kit containing 10 vials with each reagent, yellow for bacterial toxins and blue for bacterial proteins

2. The principle of TOPAS test is the detection of the presence of actively growing and dividing anaerobic pathogens which results in increased levels of their toxic metabolites in the crevicular fluid. The periodontal pathogens are producing continuously toxic metabolites [9] and some of then can be found in the crevicular fluid. They are also releasing bacterial proteins and endotoxins, which also can be found in the crevicular fluid [10]. The host subjected to bacterial aggression releases certain factors

of the immune response: antibodies, cytokines, which are responsible for the induction of inflammatory response in periodontal structures [11].

The greater the degree of the metabolic activity, the higher the concentrations of these toxins, shown by the color intensity scale of the TOPAS test. This test includes two reagents, one for measuring the concentration of bacterial toxins and the second for measuring the level of total proteins in the crevicular fluid (*Figure 2*). First the paper point imbedded in crevicular fluid is introduced in the yellow cap vial. Here the reaction of the bacterial toxins from the crevicular fluid with certain chemical colorless reagents contained in the yellow cap vial takes place. At the end of the reaction (5 minutes) the color will turn to **yellow**. The greater the concentration of bacterial toxins in the sample, the brighter yellow color will result from the reaction. The second step consists in measuring the level of total proteins (including antibodies, human serum albumin, aspartate aminotransferase, beta glucuronidase and bacterial proteins). Bacterial infection has been shown to increase the levels of these and other proteins in crevicular fluid. The second reaction of TOPAS is based on the color change produced by adding the reagent from the blue cap vial. The greater the level of total protein in the GCF sample, the brighter the assay mixture will turn to **blue**.

Based on the above-mentioned theoretical considerations we assessed the clinical and therapeutic importance of these two tests on a group of 61 subjects, all with different forms of dental crowding and gingivitis or periodontitis. The subjects are young, aged between 15 and 46 years of age. Clinical chart was done in all patients including periodontal examination before and after treatment. A number of 32 patients followed orthodontic treatment and the rest only periodontal treatment consisting in debridement and application of local antiseptic substances. BANA test was done after orthodontic treatment in case of 32 patients and after periodontal debridement in the rest of 29 patients. TOPAS test was done in all patients twice: first before any treatment and the second time after orthodontic alignment or after scaling and root planning in periodontal treated group of patients. The program used for the statistical assessment of this case control study is **MedCalc, version 7.3.0.1** [12]. The results were analyzed using *SpearmanÁs rank correlation coefficient.*

The purpose of the study is to establish if there exists any correlation between the clinical periodontal findings and the paraclinical results of TOPAS and BANA tests.

Results

The variables used for the statistical study are: The Plaque Index (PI), The Papilla Bleeding Index (PBI), the severity of crowding assessed by TSALD coefficient, level of bacterial toxins (BT) measured with TOPAS test, level of total proteins (TP) measured also with TOPAS test and the concentration of pathogen anaerobic bacteria evaluated with BANA test as negative, weak positive or positive.

We present a 31-year-old patient, female, with chronic marginal profound periodontitis (adult periodontitis).

Before treatment, TOPAS test is performed. The paper point is inserted in the periodontal pocket (*Figure 3*) and left one minute to absorb the fluid. The result (*Figure 4*) is a moderate bacterial toxicity (degree 3 on the scale).

Then the content of the blue cap vial is added and after 2 minutes the color turned to blue. The result is an intermediate concentration of the total proteins in the pocket fluid (*Figure 5*).

Those results suggest a moderate level of gingival inflammation in an evolutive state.

Figure 3. The crevicular fluid is absorbed with the paper point

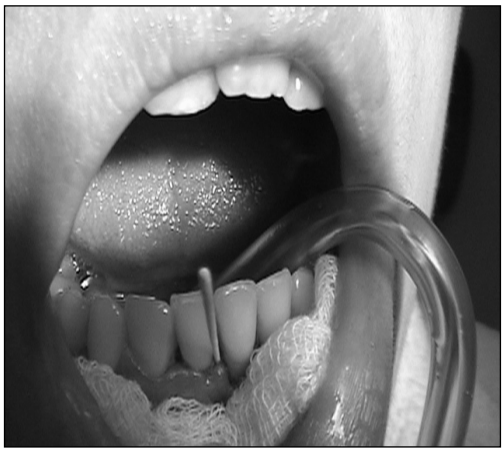


Figure 4. The result of TOPAS indicate a moderate bacterial toxicity

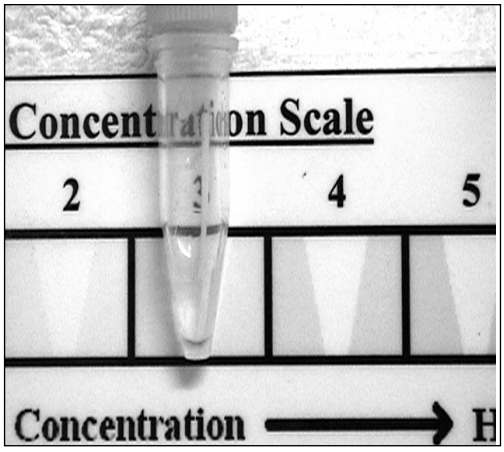
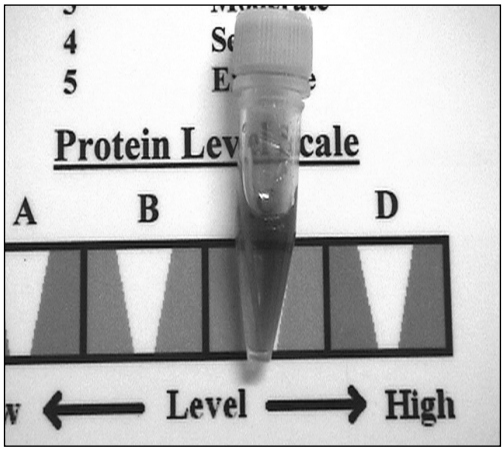


Figure 5. The level of proteins in the pocket fluid is intermediate



The subgingival plaque was removed with a Gracey curette from the lower frontal area, where the periodontal involvement is more severe and placed on the lower matrix of the BANA strip (*Figure 7*).

After the strip was incubated for 5 minutes at 55-Celsius degrees we obtained a weak positive result in all 4 samples (*Figure 6*). This result in a patient with objective signs of periodontal inflammation shows the presence of periodontal pathogen bacterial species and requires a systemic antibiotherapy.

Because on the TOPAS scale the level of total proteins is evaluated in letters from A to D we associated to each letter a number from 1 to 4 in order to ease the statistical calculation. The same was done for the BANA results, associating the value 1 instead of negative result, 2 instead of weakly positive and 3 instead of positive BANA result.

Figure 6. BANA test is weak positive

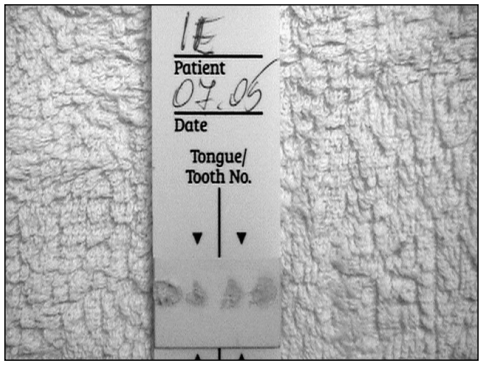


Figure 7. The BANA test strip

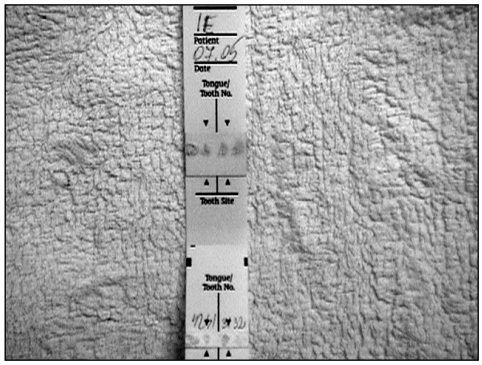


Table 1. Correlation between PBI and TOPAS findings before treatment (Spearman's rank correlation coefficient)

	PBI		**TOPAS TP**		**TOPAS BT**	
	rho	P	rho	P	rho	P
Severity of crowding	-0.275	0.0332	0.086	0.5037	-0.174	0.1785
PBI	1	< 0.00001	0.345	0.0075	0.635	< 0.0001

In *Table 1* we observe that in our study there is no statistical correlation between the severity of crowding and the degree of inflammation assessed with PBI index as well as between severity of crowding and TOPAS results. There is a statistical good correlation (almost perfect) between PBI and the level of bacterial toxins (BT), shown by **rho** index close to value 1 (which is the ideal situation) and the probability that the null hypothesis (***P***) is tending to zero. If ***P*** is lower than 0.05 it is considered that there is enough evidence of the correctitude of the results (to reject the null hypothesis). A good correlation was found also between the degree of gingival inflammation (PBI) and the level of total proteins (TP) measured with TOPAS.

The results after treatment (periodontal and orthodontic) are presented in *Table 2*.

Table 2. Correlation between PBI and TOPAS findings after treatment (Spearman's rank correlation coefficient)

	PBI		**TOPAS TP**		**TOPAS BT**	
	rho	P	rho	P	rho	P
PBI	1	< 0.00001	0.495	0.0001	0.547	< 0.0001

The same positive correlation between the severity of bleeding and TOPAS findings are found after treatment, when both values (PBI index and concentration of bacterial toxins) decreased and we deal with an improvement of the periodontal condition, shown clinically by the reduction of the bleeding tendency and paraclinically by the reduction of the level of toxicity in the crevicular fluid, measured with TOPAS test.

After testing BANA in all patients we compared the results with the severity of periodontal involvement evaluated by the type of disease. The patients included in this study were diagnosed with one of the following disease: simple chronic **gingivitis** with microbial origin (was attributed the value 1 for the statistic calculation), marginal chronic **superficial periodontitis** (was noted with value 2) and chronic marginal **profound (adult) periodontitis** (was noted with value 3).

Table 3. Correlation between BANA results and periodontal disease and plaque

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

There is a statistical positive correlation between the results of BANA test and the degree of periodontal involvement, meaning gingivitis, superficial periodontitis and profound periodontitis assessed with Spearman coefficient (*Table 3*).

Discussion

In all patients the periodontal debridement (associated or not with orthodontic alignment) led to a reduction of clinical signs of inflammation and a reduction of the bacterial toxicity level in the crevicular fluid assessed with TOPAS test. A statistical good correlation was found between the degree of gingival inflammation assessed with bleeding index (PBI) and the level of bacterial toxicity assessed by TOPAS test.

Most patients having gingivitis had BANA negative results, suggesting that the three periodontal pathogens (*Treponema denticola, Porphyromonas gingivalis and Bacteroides forsythus*) are absent or exist in low concentrations (less than 10.000 colonies forming units in the sample of subgingival plaque). A weak positive BANA result was obtained in certain patients with long-evolution gingivitis. These patients are included in a risk category and without a proper therapy they can develop superficial periodontitis and further adult periodontitis.

In most patients exhibiting superficial periodontitis the BANA results are weak positive, even negative in few cases. A weak positive BANA result in a patient that exhibits periodontal inflammatory signs shows a high risk to develop irreversible periodontal destruction. This fact is according to the prognosis and evolution of untreated superficial periodontitis. In patients with profound periodontitis (adult periodontitis) BANA results were positive or weak positive. This finding means that the periodontal destruction in most of cases is accompanied by the presence in subgingival plaque of the BANA positive anaerobic species *Treponema denticola, Porphyromonas gingivalis and Bacteroides forsythus.* Therefore, systemic antibiotherapy is required in such cases of BANA positive patients.

Conclusion

We conclude that both paraclinical exams TOPAS and BANA are quick and easy to be performed in the dental surgery. They proved good correlation with the degree of gingival inflammation and severity of the periodontal disease. Therefore, these tests help the clinician in elaborating a correct diagnosis based on current evaluation of periodontal status and to elaborate a complex periodontal treatment based on clinical and paraclinical findings. This study wishes to encourage the clinicians to use such chairside tests for the diagnosis of periodontal disease.

References

1. Sanz M, Lau L, Herrera D, Morillo JM, Silva A. Methods of detection of *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, and Tannerella forsythensis* in periodontal microbiology, with special emphasis on advanced molecular techniques: a review. *J Clin Periodontol* 2004; **31**: 1034-1047.

2. Jervùe-Storm P-M, Koltzscher M, Falk W, Dorfler A, Jepsen S. Comparison of culture and real-time PCR for detection and quantification of five putative periodontopathogenic bacteria in subgingival plaque samples. *J Clin Periodontol* 2005; **32**: 778-783.

3. Socransky SS, Haffajee AD. Periodontal microbial ecology. *Periodontology 2000*, 2005; **38**: 135-187.

4. L□esche WJ: Comparison of the benzoyl-DL-arginine-naphthylamide (BANA) test, DNA probes, and immunological reagents for ability to detect anaerobic periodontal infections due to Porphyromonas gingivalis, Treponema denticola and Bacteroides forsythus. *J. Clin Microbiol*. 1992; **30**: 427-433

5. Shimada K, Mizuno T, Ohshio K, Kamaga M, Murai S, Ito K. Analysis of aspartate aminotransferase in gingival crevicular fluid assessed by using PocketWatchTM: a longitudinal study with initial therapy. *J Clin Periodontol* 2000; **72**: 819-823.

6. Armitage GC. Analysis of gingival crevice fluid

and risk of progression of periodontits, *Periodontology 2000*, 2004; **34**: 109-119.

7. Socransky SS, Haffajee AD, Cuglini MA. Microbial complexes in subgingival plaque. *J Dent Res* 1997; **76**(special issue): abst 302.

8. Ellen RP, Galimanas VB. Spirochetes at the forefront of periodontal infections. *Periodontology 2000*, 2005; **38**: 13-32.

9. Dumitriu HT, Dumitriu S. Periodontology. (in Romanian) Ed. Via□a Medical□ Rom□neasc□ 1999; 63-65, 72-74.

10. Lindhe J, Karring T, Lang NP. Clinical Periodontology and Implant Dentistry, Blackwell Munksgaard 2003: 164-166, 211-213.

11. Vernal R, Dutzan N, Chaparro A, Puente J, Valenzuela MA, Gamonal J. Levels of interleukin-17 in gingival crevicular fluid and in supernatants of cellular cultures of gingival tissue from patients with chronic periodontitis. *J Clin Periodontol* 2005; **32**: 383-389.

12. Petrie A, Sabin C. Medical Statistics at a Glance. *Blackwell Science* 2000: 34-46, 68-69.

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