The ability of the BANA Test to detect different levels of *P. gingivalis*, *T. denticola* and *T. forsythia*

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Abstract: The aim of this study was to evaluate the ability of the BANA Test to detect different levels of *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* or their combinations in subgingival samples at the initial diagnosis and after periodontal therapy. Periodontal sites with probing depths between 5-7 mm and clinical attachment level between 5-10 mm, from 53 subjects with chronic periodontitis, were sampled in four periods: initial diagnosis (T0), immediately (T1), 45 (T2) and 60 days (T3) after scaling and root planing. BANA Test and Checkerboard DNA-DNA hybridization identified red complex species in the subgingival biofilm. In all experimental periods, the highest frequencies of score 2 (Checkerboard DNA-DNA hybridization) for *P. gingivalis*, *T. denticola* and *T. forsythia* were observed when strong enzymatic activity (BANA) was present ($p < 0.01$). The best agreement was observed at initial diagnosis. The BANA Test sensitivity was 95.54% (T0), 65.18% (T1), 65.22% (T2) and 50.26% (T3). The specificity values were 12.24% (T0), 57.38% (T1), 46.27% (T2) and 53.48% (T3). The BANA Test is more effective for the detection of red complex pathogens when the bacterial levels are high, i.e. in the initial diagnosis of chronic periodontitis.

Descriptors: DNA probes; Enzyme assays; Microbiology; Diagnosis, oral; Periodontal diseases.

Introduction

Porphyromonas gingivalis, *Treponema denticola* and *Tannerella forsythia* have been implicated in periodontal disease by cultural,[1] immunological[2] and DNA probe[3] studies. These species are Gram-negative anaerobes which, *in vivo*, have an enzyme capable of hydrolyzing the synthetic trypsin substrate, N-benzoyl-DL-arginine-2-naphthylamide (BANA). Based on this enzymatic profile, Loesche *et al.*[4] (1990) described a microbiological test denominated the BANA Test, which uses a chromophore added to a synthetic peptidase as a substrate (Benzoyl-DL-Arginine-Naphthylamide). The presence of at least 10^4 cells of *T. denticola*, *P. gingivalis* and/or *T. forsythia* results in a blue color on the card. These considerations indicate that detection of the BANA enzyme in plaque samples most likely reflects the presence of these pathogens in the samples.[5]

In 1994, Socransky *et al.*[6] (1994) described the Checkerboard DNA-DNA hybridization microbiological test. This technique includes the

use of whole genomic DNA probes and allows the identification and the quantification of as many as 45 bacterial species in up to 28 clinical samples per test. This technique has showed better results in comparison with other microbiological tests.[7-11] Socransky *et al.*[3] (1998) described the presence of five, and subsequently six microbial groups[12] within subgingival biofilm. A group of species denominated the red complex consisted of three species: *P. gingivalis, T. forsythia* and *T. denticola*. This complex is strongly related to probing depth (PD) and bleeding on probing.[3,12]

The BANA Test has been used in several studies.[13-17] The results of the BANA Test have been previously compared with other microbiological techniques, such as ELISA (enzyme-linked immunoabsorbent assay) reaction[18] and PCR.[19] However, these studies compared only the bacterial enzymatic activity with the presence or absence of the microorganisms. Additionally, it is not clear whether a BANA-positive reaction is associated with isolated or simultaneous presence of the red complex species. The aim of this study was to evaluate the ability of the BANA Test to detect different levels of *P. gingivalis*, *T. denticola* and *T. forsythia* or their combinations in subgingival samples at the initial diagnosis and after periodontal therapy.

Materials and Methods

Study population and periodontal procedures

Sixty subjects with at least 30 years of age and 15 teeth, diagnosed with generalized chronic periodontitis were selected for the study. Measurements (mm) of PD and clinical attachment level (CAL) and the presence (1) or absence (0) of plaque, gingival inflammation and bleeding on probing were obtained at six sites per tooth by two calibrated examiners (inter-examiner, Kappa = 92% and intra-examiner, Kappa = 91%-93%) using a manual periodontal probe (Hu-friedy Mfg Co. Inc. Chicago, IL, USA). All subjects included in this study presented at least 6 interproximal, non contiguous sites, distributed in different quadrants, with 5-7 mm of PD and 5-10 mm of CAL. Exclusion criteria were: tobacco; pregnancy; lactation; orthodontic devices; periodontal or antibiotic therapy in the previous 6 months; any systemic condition that could affect the progression of periodontal disease; need for antibiotic coverage for routine dental therapy. All subjects signed an Informed Consent, which was previously approved by the Institutional Committee for Research Involving Human Subjects.

Periodontal therapy was conducted by two trained periodontists. Scaling and root planing (SRP) were performed in six visits of 1 hour each, in a period of 21 days using manual instruments (Hu-friedy Mfg Co. Inc. Chicago, IL, USA). All participants were given toothbrushes, dental floss and dentifrice with triclosan (Colgate Total®, Anakol Ind. Com. Ltda. – Kolynos do Brasil – Colgate Palmolive Co., São Bernardo do Campo, SP, Brazil).

Microbiological examination

Sample collection

After the removal of supragingival plaque, subgingival plaque samples were collected from the bottom of six interproximal periodontal pockets. These previously selected sites were non contiguous and presented PD of 5-7 mm and CAL of 5-10 mm. Sterilized mini-five (#11/12) Gracey curettes (Hu-friedy Mfg Co. Inc. Chicago, IL, USA) were inserted in the base of the pocket, and the subgingival plaque was collected. The sample was divided in two portions, one designated for the BANA Test and the other one for the Checkerboard DNA-DNA hybridization. The microbiological analysis was done in four experimental periods: initial diagnosis (T0), after SRP (T1 – immediately after the 6th visit of SRP), 45 days (T2) and 60 days after the 6th visit of SRP (T3).

BANA

The BANA Test (Knöwell Therapeutic Technologies Inc., Toronto, Ontario, Canada) was done as described by Loesche *et al.*[4] (1990). Results were registered as score 0 - negative result (no blue color); score 1 - weak-positive result (faint blue color) and score 2 - strong-positive result (definite blue color). The weak enzymatic reaction (score 1) corresponded to about 10^4-10^5 colony forming units and the strong enzymatic reaction (score 2) corresponded to about 10^6 or over 10^6 colony forming units.

Checkerboard DNA-DNA hybridization

Counts of three bacterial species were determined in each sample, using the Checkerboard DNA-DNA hybridization technique[6] as previously described by Matarazzo *et al*. (2008).[8] The assay adjusted the sensitivity of the assay to permit detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe. Signals were converted to absolute counts by comparison with the standard lanes on the membrane. Score 0 was registered when there was no signal detection; score 1 when the signal was less intense than the control at 10^5 cells; score 2 similar to 10^5 cells; score 3, between 10^5 and 10^6 cells; score 4 similar to 10^6 cells and score 5, over 10^6 cells.

Statistical analysis

The Checkerboard DNA-DNA hybridization scores were submitted to a new codification to allow a better comparison with the BANA Test results. The score 0 was maintained as a negative result ($\leq 10^4$ bacteria), scores 1 and 2 were reclassified as score 1 ($10^4 - 10^5$ bacteria) and scores 3, 4 and 5 were reclassified as score 2 ($> 10^5$ bacteria). The second new codification was applied to both diagnostic tests to estimate sensitivity and specificity values. The score 0 represented negative results and the score 1 positive results, including scores 1, 2, 3, 4 and 5 of the Checkerboard DNA-DNA hybridization and scores 1 and 2 of the BANA Test. The frequencies of BANA Test results according to the different bacteria levels determined by Checkerboard DNA-DNA hybridization were analyzed by Chi-Square test. The sensitivity and specificity values of the BANA Test related to Checkerboard DNA-DNA hybridization were determined by Chi-Square test. The significance level was established at 5% ($p < 0.05$) and the data were analyzed using software Bioestat® 5.0 (Pará University, Belém, Pará, Brazil) and SPSS® 11.5 (IBM Company, Chicago, Illinois, USA).

Results

Out of the sixty subjects included in the study, seven did not complete the experimental phase. Fifty-three subjects (34 females and 19 males; 30 to 61 years of age) participated in this study. The full-mouth mean clinical parameters are summarized in Table 1.

The possible combinations between each individual bacterial species related to each pattern of enzymatic activity of the BANA Test are presented in Table 2. In all experimental periods, the highest frequencies of score 2 (Checkerboard DNA-DNA hybridization) for *P. gingivalis*, *T. denticola* and *T. forsythia* were observed when strong enzymatic activity was present (Chi-Square test, $p < 0.05$). The best agreement was at time T0 [*P. gingivalis* (frequency = 233), *T. denticola* (frequency = 197) and *T. forsythia* (frequency = 238)]. On the other hand, the absence of enzymatic activity was not followed by absence of the DNA bacterial detection at all time points. In T1, T2 and T3 the score 0 of *T. denticola* was the most frequent result associated with the score 0 of the BANA Test. In experimental periods T2 and T3, detection of BANA Test score 0 was associated with high frequencies of Checkerboard DNA-DNA hybridization score 2, for *T. forsythia* and *P. gingivalis*.

The BANA results were compared with the presence of each of the red complex species detected by Checkerboard DNA-DNA hybridization (Table 3). The distribution of the scores showed an association for *P. gingivalis* in all experimental periods, for *T. denticola* in T1 and T2; and for *T. forsythia* in T1, T2 and T3. A significant association between the BANA Test and Checkerboard DNA-DNA hybridization was observed for red complex detection in the initial diagnosis (T0). The sensitivity values ranged between 94.61% - 95.24% for the species separately and was 95.54% considering the red complex. At 60 days after SRP (T3) there was a decrease in sensi-

Table 1 - Mean full-mouth clinical parameters of the 53 subjects at initial diagnosis (T0) of chronic periodontitis.

Clinical parameter	Mean ± Standard deviation
Probing depth (mm)	3.66 ± 1.77
Clinical attachment level (mm)	4.15 ± 2.17
% of sites with	
Plaque	82.30 ± 12.40
Gingival inflammation	35.20 ± 19.40
Bleeding on probing	59.20 ± 19.80

Table 2 - Evaluation of BANA Test results according to the bacteria levels determined by Checkerboard DNA-DNA hybridization in the four time points.

Time	BANA Test scores	Checkerboard DNA-DNA hybridization scores								
		P. gingivalis			*T. denticola*			*T. forsythia*		
		0	1	2	0	1	2	0	1	2
T0	0	4	3	11	4	2	12	2	1	15
	1	7	7	30	12	7	25	6	3	35
	2	13	10	233	30	29	197	13	5	238
	χ^2 (p value)	$p < 0.0001$			$p < 0.05$			$p < 0.05$		
T1	0	47	61	44	92	38	22	75	31	46
	1	12	38	24	37	30	7	24	20	30
	2	15	24	53	37	28	27	20	24	48
	χ^2 (p value)	$p < 0.001$			$p < 0.001$			$p < 0.001$		
T2	0	35	50	41	58	37	31	43	32	51
	1	19	20	36	25	21	29	22	14	39
	2	9	39	69	39	32	46	21	22	74
	χ^2 (p value)	$p < 0.001$			$p = 0.089$ (NS)			$p < 0.01$		
T3	0	30	61	72	65	49	49	39	39	85
	1	11	26	40	28	26	23	14	20	43
	2	2	23	53	28	13	37	7	15	56
	χ^2 (p value)	$p < 0.01$			$p < 0.05$			$p < 0.05$		

T0: initial diagnosis; T1: immediately after scaling and root planing; T2: 45 days and T3: 60 days after scaling and root planing. NS= not significant ($p > 0.05$); χ^2 = Chi-Square test. Scores: 0 = < 10^4 cells, 1 = 10^4 - 10^5 cells, 2 = ≥ 10^6 cells.

Table 3 - Sensitivity and specificity of BANA Test in the 4 experimental periods. **[continued on next page]**

Checkerboard DNA-DNA hybridization scores											
Time	BANA Test scores	*P. gingivalis* (P.g.)		*T. denticola* (T.d.)		*T. forsythia* (T.f.)		Red complex (P.g. and T.d. and T.f.)		P.g. and/or T.d. and/or T.f.	
		0	1	0	1	0	1	0	1	0	1
T0	0	4	14	4	14	2	16	6	12	2	16
	1	20	280	42	258	19	281	43	257	14	286
χ^2 (p value)		5.89 (0.015)		NS		NS		4.70 (0.03)		NS	
Sensitivity		95.24%		94.85%		94.61%		95.54%		94.71%	
Specificity		16.67%		8.7%		9.52%		12.24%		12.50%	
T1	0	47	105	92	60	75	77	105	47	37	115
	1	27	139	74	92	44	122	78	88	20	146
χ^2 (p value)		9.55 (0.002)		8.09 (0.004)		17.67 (0.000)		15.85 (0.0001)		8.15 (0.004)	
Sensitivity		56.97%		60.53%		61.31%		65.18%		55.94%	
Specificity		63.51%		55.42%		63.03%		57.38%		64.91%	

T0: initial diagnosis; T1: immediately after scaling and root planing; T2: 45 days and T3: 60 days after scaling and root planing. Scores: 0 = ≤ 10^4 cells (negative result), 1 = > 10^4 cells (positive result). NS = not significant ($p > 0.05$); χ^2 = Chi-Square test.

Table 3 [continued] - Sensitivity and specificity of BANA Test in the 4 experimental periods.

Time	BANA Test scores	Checkerboard DNA-DNA hybridization scores									
		P. gingivalis (P.g.)		*T. denticola* (T.d.)		*T. forsythia* (T.f.)		Red complex (P.g. and T.d. and T.f.)		P.g. and/or T.d. and/or T.f.	
		0	1	0	1	0	1	0	1	0	1
T2	0	35	91	58	68	43	83	62	64	27	99
	1	28	164	64	128	43	149	72	120	17	175
χ^2 (p value)		8.34 (0.004)		5.19 (0.023)		5.31 (0.021)		4.27 (0.039)		10.09 (0.001)	
Sensitivity		64.31%		65.31%		64.22%		65.22%		63.87%	
Specificity		55.56%		47.54%		50.0%		46.27%		61.36%	
T3	0	30	133	65	98	39	124	69	94	25	138
	1	13	142	56	99	21	134	60	95	6	149
χ^2 (p value)		6.82 (0.009)		NS		5.59 (0.018)		NS		11.87 (0.001)	
Sensitivity		51.64%		50.25%		51.94%		50.26%		51.91%	
Specificity		69.77%		53.72%		65.0%		53.48%		80.64%	

T0: initial diagnosis; T1: immediately after scaling and root planing; T2: 45 days and T3: 60 days after scaling and root planing. Scores: 0 = ≤ 10^4 cells (negative result), 1 = > 10^4 cells (positive result). NS = not significant ($p > 0.05$); χ^2 = Chi-Square test.

tivity for the species separately (50.25% - 51.94%), and was 50.26% for the red complex. Considering that the BANA Test will be positive if any of the three red complex species are present in according with the levels of detection, the last column to the right of Table 3 shows the relationship between a positive Checkerboard DNA-DNA hybridization result for at least one species and the BANA Test results. The highest and the lowest sensitivity levels were observed at initial diagnosis (T0) and 60 days after SRP (T3), respectively.

Discussion

In the present study, there was a good correlation between the BANA scores 0, 1 and 2 and the DNA scores 0, 1 and 2 for each of the three bacterial species evaluated at the initial time point (T0, Table 2). This was especially true when the results were condensed to presence or absence of enzyme activity and the presence or absence of the DNA from red complex species (Table 3). In this case, at initial diagnosis (T0) the values for sensitivity were high (between 94.61% and 95.24%). Eighty six percent (257/300) of BANA positive samples contained all three red complex species and 95.3% (286/300) contained one or more of the three species. In agreement with our data, others have also found a good correlation between DNA probes and the BANA Test in untreated subjects, i.e comparable with time T0. Loesche *et al.*[5] (1992) compared the BANA Test to commercially available whole chromosomal DNA probes to *P. gingivalis* and *T. denticola* (no probe to *T. forsythia* was available), and immunological reagents in their ability to detect the red complex species in patients scheduled for periodontal treatment. When the DNA probes were used as the primary reference the BANA Test had a sensitivity of 90% and an accuracy of 83%. Takaishi *et al.*[19] (2003) found a significant correlation between BANA activity and the presence of the red complex species using a PCR method and commercially obtained probes.

No one has reported on comparisons between the DNA probes and the BANA Test in plaque samples taken after treatment as was done in the present study. At the time periods post-therapy (T1, T2 and T3) the correlations between BANA activity and the DNA detection were also significant for most analyses (Table 3). However, it should be noted that the good sensitivity values observed at T0 were reduced over the course of the study, showing values lower than 70%. This was primarily due to the DNA detection of the red complex species in BANA nega-

tive samples. Sixty six percent (12/18) of the BANA negative sites contained the red complex species and 88.8% (16/18) contained one or more of the three species. This might reflect that the DNA probes are more sensitive than the BANA Test in detecting low levels of these pathogens. Alternatively, it could also mean that the probes were cross-reacting with one or more of the over 500 species that are known to inhabit the oral flora.[20] However, this last possibility is unlikely since it has been reported by Socransky *et al*.[21] (2004) that whole genomic DNA probes for *T. forsythia*, *P. gingivalis* and *T. denticola* showed essentially no cross-reactions to 80 oral bacterial species at the level > 10% of the homologous probe signal.

The specificity values were low in all experimental periods (Table 3). About 13% of the samples at T0 had a BANA positive score of 1 or 2 in the absence of detectable red complex species. This number increased to 24% at T1, and 23% at T2 but declined to 18% at T3. Thus it would appear that about 13 to 24% of the BANA activity in plaque samples may be due to unidentified BANA positive species. The BANA activity in subgingival plaque samples has been associated with the red complex species, because in the development of the BANA Test, *P. gingivalis*, *T. denticola* and *T. forsythia* were the only species out of over 50 oral microorganisms tested that were always BANA positive.[4] Nevertheless, it is likely that other BANA positive species exist in the oral cavity. A recent analysis of oral microbial ribosomal sequences has found new bacterial species possibly associated with chronic periodontitis[22] whose enzymatic profile has not yet been determined.

It should also be noted that the specificity of the BANA Test increased after SRP, especially at T3 (80.64%), when the number of healthy sites also increased. These findings suggest that the inclusion of only diseased periodontal sites (PD 5-7 mm) in this study might have influenced the results for specificity. Therefore, studies using the same methodology as the present one and including healthy periodontal sites could help to clarify this issue.

Conclusion

The BANA Test was shown to be an effective technique for the detection of the red complex pathogens in the initial diagnosis of chronic periodontitis; however, its application for post-therapy monitoring has limitations.

References

1. Dzink JL, Socransky SS, Haffajee AD. The predominant cultivable microbiota of active and inactive lesions of destructive periodontal diseases. J Clin Periodontol. 1988 May;15(5):316-23.
2. Grossi SG, Genco RJ, Machtei EE, Ho AW, Koch G, Dunford R, *et al*. Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. J Periodontol. 1995 Jan;66(1):23-9.
3. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent Jr RL. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998 Feb;25(2):134-44.
4. Loesche WJ, Bretz WA, Kerschensteiner D, Stoll J, Socransky SS, Hujoel P, *et al*. Development of a diagnostic test for anaerobic periodontal infection based on plaque hydrolysis of Benzoyl-DL-Arginine-Naphthlamide. J Clin Microbiol. 1990 Jul;28(7):1551-9.
5. Loesche WJ, Lopatin DE, Giordano J, Alcoforado G, Hujoel P. 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 Feb;30 (2):427-33.
6. Socransky SS, Smith C, Martin L, Paster BJ, Dewhirst FE, Levin AE. "Checkerboard" DNA-DNA Hybridization. Biotechniques 1994 Oct;17(4):788-92.
7. Colombo AP, Teles RP, Torres MC, Souto R, Rosalem J, Mendes MC, *et al*. Subgingival microbiota of Brazilian subjects with untreated chronic periodontitis. J Periodontol. 2002 Apr;73(4):360-9.
8. Matarazzo F, Figueiredo LC, Cruz SE, Faveri M, Feres M. Clinical and microbiological benefits of systemic metronidazole and amoxicillin in the treatment of smokers with chronic periodontitis: a randomized placebo-controlled study. J Clin Periodontol. 2008 Oct;35(10):885-96.
9. Ximenez-Fyvie LA, Haffajee AD, Thompson M, Torresyap G, Socransky SS. The effect of repeated professional supragengival plaque removal on the composition of the supra- and sub-

gingival microbiota. J Clin Periodontol. 2000 Sep;27(9):637-47.

10. Leonahrdt A, Bergstrom C, Lekholm U. Microbiologic diagnostics at titanium implants. Clin Implant Dent Relat Res. 2003 Dec;5(4):226-32.
11. Papapanou PN, Mandianos PN, Dahlén G, Sandros J. "Checkerboard" *versus* culture: a comparison between two methods for identification of subgingival microbiota. Eur J Oral Sci. 1997 Oct;105(5 Pt 1):389-96.
12. Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. Periodontol 2000. 2002 Jan;28:12-55.
13. Figueiredo LC, Toledo BE, Salvador SL. The relationship between place BANA reactivity and clinical parameters in subjects with mental disabilities. Spec Care Dentist. 2000 Oct;20(5):195-8.
14. Lee Y, Straffon LH, Welch KB, Loesche WJ. The transmission of anaerobic periodontopathic organisms. J Dent Res. 2006 Feb;85(2):182-6.
15. Grisi DC, Salvador SL, Figueiredo LC, Souza SL, Novaes AB, Grisi MF. Effect of a controlled-release chlorhexidine chip on clinical and microbiological parameters of periodontal syndrome. J Clin Periodontol. 2002 Oct;29(10):875-81.
16. Vergani SA, Barbosa e Silva E, Vinholis AH, Marcantonio RAC. Systemic use of metronidazole in the treatment of chronic periodontitis: A pilot study using clinical, microbiological, and enzymatic evaluation. Braz Oral Res. 2004 Apr-Jun;18(2):121-7.
17. Peruzzo DC, Salvador SL, Sallum AW, Nogueira-Filho GR. Flavoring agents present in a dentifrice can modify volatile sulphur compounds (VSCs) formation in morning bad breath. Braz Oral Res. 2008 Jul-Sep;22(3):252-7.
18. Bretz WA, Eklund SA, Radicchi R, Schork MA, Schork N, Schottenfeld D, *et al*. The use of a rapid enzymatic assay in the field for the detection of infections associated with adult periodontitis. J Public Health Dent. 1993 Dec;53(4):235-40.
19. Takaishi Y, Morri H, Miki T. The benzoyl-DL argininanaphthylamide (BANA) test and polymerase chain reaction measurement of pathogenic bacteria can assess the severity of periodontal disease. Int J Tissue React. 2003;25(1):19-24.
20. Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA, *et al*. Bacterial diversity in human subgingival plaque. J Bacteriol. 2001 Jun;183(12):3770-83.
21. Socransky SS, Haffajee AD, Smith C, Martin L, Haffajee JA, Uzel NG, *et al*. Use of checkerboard DNA-DNA hybridization to study complex microbial ecosystems. Oral Microbiol Immunol. 2004 Dec;19(6):352-62.
22. Kumar PS, Griffen AL, Barton JA, Paster BJ, Moeschberger ML. New bacterial species associated with chronic periodontitis. J Dent Res. 2003 May;82(5):338-44.