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Influence of highly-active antiretroviral therapy on the subgingival biofilm in HIV-infected patients

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Abstract

Aim: Highly-active antiretroviral therapy (HAART) has been associated with alterations in subgingival biofilm and periodontal disease. The purpose of the present study was to investigate the association between different HAART regimens and the prevalence of periodontal pathogens in HIV-infected patients with chronic periodontitis.

Methods: Subgingival periodontal pathogens were determined by a DNA chip microarray in a case series in 14 HIV-infected patients receiving HAART with different drug combinations: protease inhibitor (PI)-based HAART versus non-nucleoside reverse transcriptase inhibitor (NNRTI)-based HAART. A statistical analysis was conducted to determine whether specific HAART regimens were associated with 10 periodontal pathogens using odds ratios (OR).

Results: At baseline and after treatment, the patients did not show significant clinical and immunological differences. In general, the highest OR for the prevalence of periodontal pathogens were found in the PI HAART group for *Actinomyces viscosus* (*A. viscosus*) (OR: 303), *Campylobacter rectus* (OR: 90), and *Treponema denticola* (OR: 25). In the NNRTI HAART group, higher OR were documented for *Fusobacterium nucleatum* (OR: 56) and *Eikenella corrodens* (OR: 25). The association between *A. viscosus* and PI HAART was statistically significant ($P = 0.03$).

Conclusions: The results demonstrated statistical associations between subgingival bacteria and antiretroviral drug therapies. Further investigation on the clinical significance and underlying mechanisms are needed to support these findings.

Introduction

Microbiological studies in HIV-infected patients have been conducted since the beginning of the worldwide epidemic, but less is known about antiretroviral drug influences on periodontal pathogens. The scientific investigation of the subgingival biofilm in HIV-infected patients can be divided into different stages. First, investigations before 1996, the time of the development of highly-active antiretroviral therapy (HAART), when periodontal pathogens were studied in the natural progression of HIV infection; second, investigations after 1996. Pre-HAART results have demonstrated a high prevalence of periodontal diseases, especially acute forms, such as necrotizing ulcerative gingivitis and periodontitis, accompanied by high levels of periodontal and atypical microbiota.[1,2] HAART has shifted HIV infection into a chronic disease, with a significantly decreased mortality and improved quality of life.[3,4] This includes a changing prevalence in HIV-related and AIDS-indicating diseases of the oral cavity, such as Kaposi sarcoma, but also new HIV-related oral lesions, such as oral warts.[5,6] However, the overall preventive fraction of HIV-related oral lesions in patients undergoing protease inhibitor (PI)-based HAART is approximately 30%.[7] Bacterial and fungal infections, in particular, decreased significantly under HAART, whereas oral lesions of viral or autoimmune origin, as well as neoplasia, have been found to have a significant impact in some populations.[8] Scientific evidence of the role of specific antiretroviral drugs, such as the key medicine protease

inhibitors and non-nucleoside reverse transcriptase inhibitors (NNRTI), is inconsistent. Protease inhibitors are widely investigated, demonstrating a significant effect in preventing HIV-related oral lesions compared to PI-sparing drug regimens.[9] For instance, several clinical and *in vitro* investigations have demonstrated a direct, immune reconstitution-independent effect of PI on the prevalence of oral candidiasis.[10] In a Brazilian cohort, HIV-infected patients with chronic periodontitis undergoing HAART showed even less periodontal inflammation and destruction, as compared to a HIV-seronegative control group with chronic periodontitis. This effect was also obvious in patients on semi-optimal HAART with immunosuppression and detectable viremia, indicating that there might be a HAART-dependent protective effect in the clinical course of periodontal diseases, as well as general immune reconstitution. The study also revealed a significant difference in the subgingival bacterial load and elevated levels of microbiota of the red and orange complex in the HIV-seronegative control group for the benefit of HAART patients.[11] However, it was also shown that reverse transcriptase inhibitors (RTI) are able to compromise periodontal health,[12] and epidemiological studies have shown significantly more HIV-related oral lesions in patients on PI HAART compared to those taking an NNRTI HAART, with twice the amount of oral lesions.[13]

The findings indicate an influence of HAART drug regimens in the development of HIV-related oral lesions, particularly bacterial infections. Microbiological investigations of the subgingival biofilm composition in HIV-infected patients undergoing HAART have demonstrated clear differences compared to an HIV-seronegative control group,[11] yet no data are available concerning the potential influence of different antiretroviral key drugs (PI, NNRTI) on periodontal pathogens, restricting the development of a working hypothesis. Therefore, the aim of this preliminary investigation was to analyze differences in the subgingival profile of HIV-infected patients with chronic periodontitis on supportive periodontal therapy (SPT) undergoing different HAART regimens to generate a working hypothesis for a prospective clinical study.

Material and methods

Patients attending an outpatient HIV oral health care center in Berlin, Germany, who were undergoing HAART for at least 6 months, and who had chronic periodontitis, were asked to participate in the study. They were recruited within a time slot of 12 months, meeting the primary inclusion criteria (HIV-1 infection and NNRTI or PI as a key drug of HAART; $n = 92$). Periodontal disease was defined as a minimum of four teeth with periodontal probing pocket depths ≥4 mm.[14] Periodontal examination was performed using a PCP 12 periodontometer (HuFridey, Chicago, IL, USA) by a calibrated examiner (intra-examiner reliability: $\kappa = 0.91$). After screening for the secondary inclusion criteria (chronic periodontitis with at least 4 teeth with probing pocket depths of ≥4 mm and younger than 45 years of age), 14 patients met the initial inclusion criteria for participation. Of these, 11 concluded the study. Exclusion criteria were the presence of other systemic disorders associated with chronic periodontitis, such as diabetes; patients taking antibiotics at least 3 months prior to the examinations, at baseline, and after treatment; and a lack of compliance concerning SPT and informed consent, respectively. The patients were periodontally treated according to a standard therapy protocol. Scaling and root planing was performed by a single operator, and SPT by a single dental hygienist, respectively. After three appointments of an initial hygiene phase with professional tooth cleaning and oral hygiene instructions at 2-week intervals, the baseline measurement of clinical parameters of periodontal, hematological, and viremic data was done (Table 1). Subsequently, scaling and root planing was performed in all teeth with periodontal probing pocket depths ≥4 mm, followed by SPT with an appointment schedule according to the individual risk profile. Approximately 10.6 months after scaling and root planing and 3 months prior to study commencement, patients were rescreened for antibiotics; three patients were excluded from the study. Clinical characteristics were rerecorded, 10 periodontal pathogens were detected by a DNA chip microarray, and statistical analysis was performed (Figure 1).

Measurement of periodontal pathogens

The detection of periodontal pathogens was executed with ParoCheck 10 (Greiner BioOne GmbH, Frickenhausen, Germany). According to the recommendations of Haffajee and Socransky[15], biofilm samples were taken for 15 s in the four deepest pockets with sterile paper tips. Twenty-eight unpooled samples in seven patients with NNRTI HAART, and 16 unpooled samples in four patients with PI HAART, were thus obtained from sites with previous scaling and root planing. They were transferred into sterile collection tubes, and transported to the microbiological lab at room temperature. The test principle of the DNA chip microarray was based on the detection of the pathogen-specific 16S rRNA gene. The bacterial DNA was extracted, and subsequently, parts of the 16S-rRNA-coded gene were amplified in the presence of fluorescence-labelled primer using polymerase chain reaction (PCR). The labelled and amplified parts were then hybridized to pathogen-specific DNA probes from the areas of the 16S rRNA gene that were spotted to the DNA chip. The measurement of bound DNA was carried out with microarray scanners. The limit of

Table 1. Clinical characteristics of patients with NNRTI HAART and PI HAART at baseline and after treatment

Time	Patient group	Age (years) (95% CI)	Sex	No. samples	Smoking habit	Median months of SPT (95% CI)	Median no. teeth (95% CI)	Median no. pockets, 4–6 mm (95% CI)	Median no. pockets >6 mm (95% CI)	Median CD4 counts (95% CI)	Median viral load (95% CI)
Baseline	NNRTI HAART	40.0 (33.8, 42.8)	Male	28	57%	11.0 (2.8, 23.0)	27.0 (23.3, 28.4)	8.0 (5.1, 12.3)	0 (0.0, 1.2)	360 (0.0, 942.7)	28,060 (0.0, 89,246)
After treatment							27.0 (23.3, 28.4)	2.0 (0.0, 4.6)	0 (0, 0)	395.5 (165.9, 665.6)	0.00 (0.0, 141,158)
Intragroup comparison (*P*-value)	–	–	–	–	–	–	1.0	0.01*	1.0	1.1	0.9
Baseline	PI HAART	35.0 (30.8, 40.7)	Male	16	50%	10.0 (2.3, 17.8)	27.5 (24.8, 29.3)	7.5 (3.6, 10.4)	0 (0, 0)	518 (37.6, 1178.0)	0.0 (0.0, 152,009)
After treatment							27.5 (23.7, 29.8)	2.0 (0.0, 4.6)	0.0 (0.0, 1.0)	615 (136.6, 950.0)	0 (0, 0)
Intragroup comparison (*P*-value)*	–	–	–	–	–	–	1.0	0.1	1.0	0.8	0.8
Intergroup comparison (*P*-value)	–	0.4	1.0	–	0.9	0.9	0.6/0.6#	0.6/0.9	1.0/1.0#	0.4/0.2	0.6/1.0

*Intragroup comparison statistically-significant difference ($P < 0.05$), #$P > 0.05$; first *P*-value: NNRTI HAART *vs* PI HAART at baseline; second *P*-value: NNRTI HAART *vs* PI HAART after treatment.
All data presented as medians, except for sex and smoking habits. CD4 counts in cells/μL blood; viral load in RNA copies/mL blood.
CI, confidence interval; HAART, highly-active antiretroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; SPT, supportive periodontal therapy.

detection of the ParoCheck 10 detecting periodontal pathogens was approximately 1476 copies per PCR (minimum: *Prevotella intermedia* [*P. intermedia*] 540 copies; maximum: *Actinomyces viscosus* [*A. viscosus*] 6013 copies). Tested pathogens were represented by the following complexes: red (*Tannerella forsythia* [*T. forsythia*], *Porphyromonas gingivalis* [*P. gingivalis*], *Treponema denticola* [*T. denticola*]), orange (*Campylobacter rectus* [*C. rectus*], *Fusobacterium nucleatum* [*F. nucleatum*], *Parvimonas micra* [*P. micra*], *P. intermedia*), and green (*Eikenella corrodens* [*E. corrodens*]. *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) exists in several serotypes (a–e). Serotype a was assigned to the green complex, whereas serotype b was not allocated to any complex.[16] *A. actinomycetemcomitans* serotyping was not performed in this investigation. As a clinical investigation of the periodontal distribution of serotypes in a German population showed, *A. actinomycetemcomitans* serotype b was the most prevalent strain;[17] therefore, *A. actinomycetemcomitans*, as well as *A. viscosus* were classified as pathogens that were not related to periodontal complexes in the present study.

Statistical analysis

Statistical analysis was calculated with Prism4 for Macintosh (Graphpad Software, San Diego, CA, USA). The primary outcome measure was the action of antiretroviral drug therapy on subgingival bacteria. Odds ratios (OR) for different HAART regimens were calculated with contingency tables. Statistical significance was determined using Fisher's exact test, with $P < 0.05$. An intergroup comparison of clinical characteristics of the patients at baseline and after treatment was performed using the Mann–Whitney *U*-test; paired observations were analyzed using Wilcoxon signed rank test.

Ethics

The study was conducted to the provisions of the Declaration of Helsinki, including informed consent of the patients. The study was approved by the ethical review board of Witten/Herdecke University, Germany (16/2009).

Results

The clinical characteristics of the patients at baseline and after treatment are summarized in Table 1. They demonstrated neither statistically significant differences concerning demographic (age, sex), behavioral (smoking habits), periodontal (teeth with periodontitis, number of teeth, and duration of SPT) nor hematological and viremic (CD4 counts and viral load) baseline data ($P > 0.05$). No significant changes were observed in these parameters after treat-

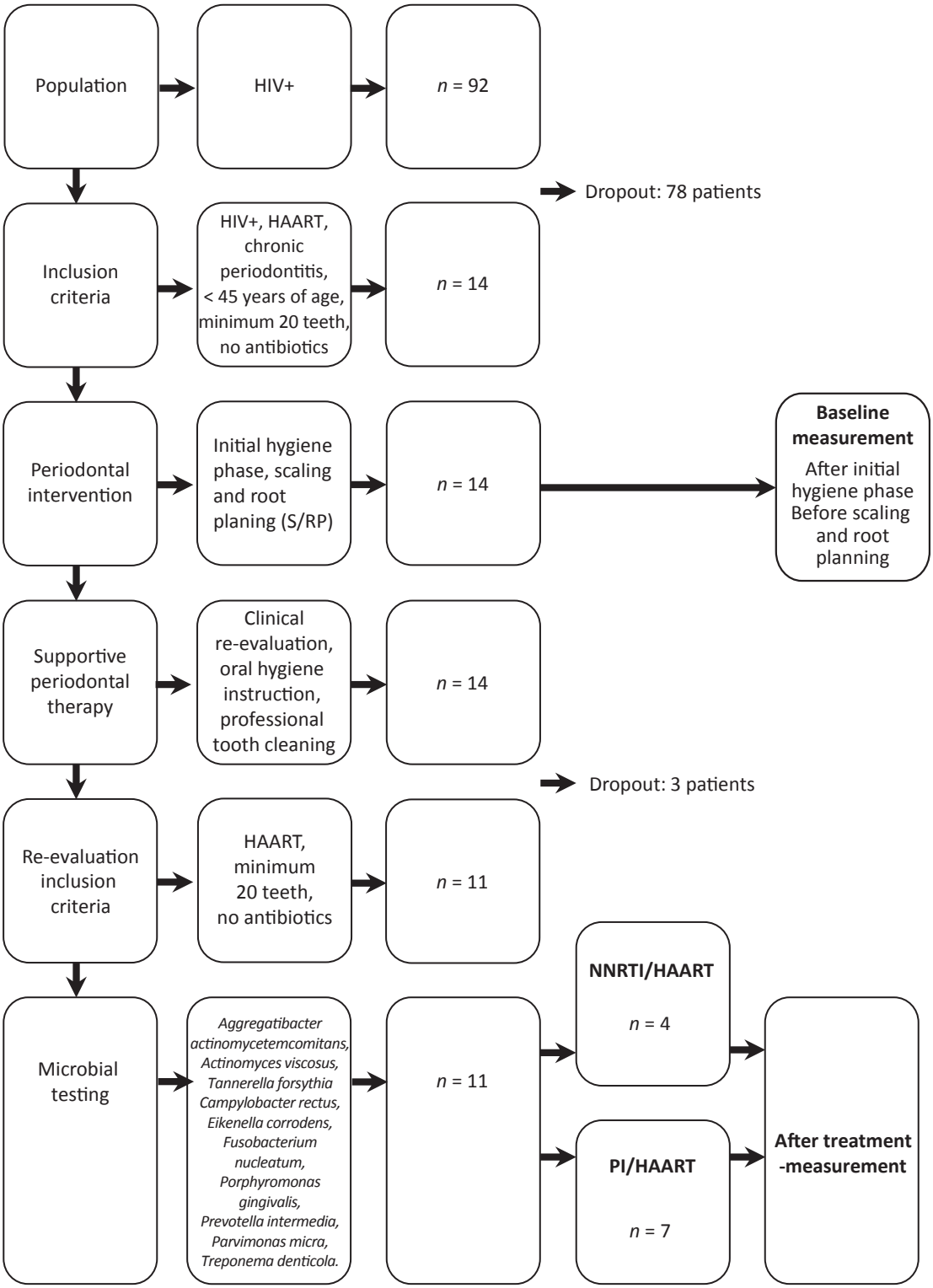

Figure 1. Study design and flow of patients during the study. HAART, highly-active antiretroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

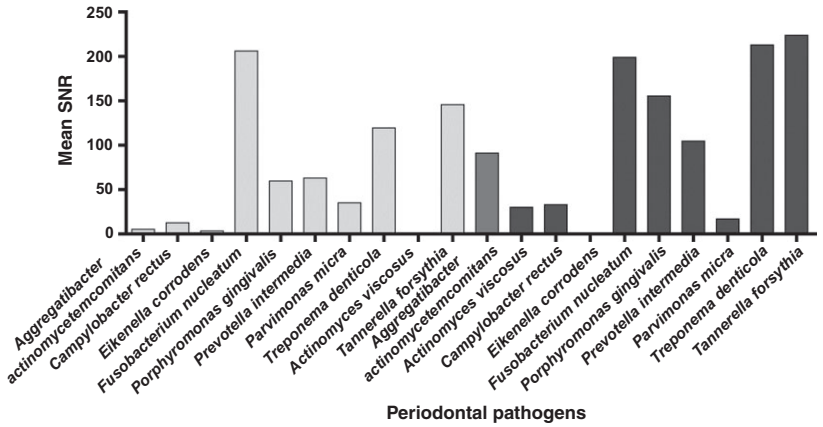

Figure 2. Distribution of periodontal pathogens in signal-to-noise ratio (SNR), grouped by non-nucleoside reverse transcriptase inhibitor highly-active antiretroviral therapy (HAART) (grey bars) and protease inhibitor HAART (black bars).

ment, except for the intragroup comparisons of the number of teeth with periodontal probing pocket depths between 4 and 6 mm ($P < 0.05$). The patients' HAART regimens consisted of first-line therapies, including an NNRTI or a PI in combination with two RTI. One patient who received triple RTI combination therapy at recruitment was supplemented an NNRTI on short notice. In general, the highest prevalence was evident for pathogens of the red and orange complexes, but there was no statistically significant difference between NNRTI HAART and PI HAART ($P > 0.5$) (Figure 2). Some periodontal pathogens showed remarkable OR when compared with the different HAART regimens. In the PI HAART group, high OR were found when compared with NNRTI HAART for *A. viscosus* (OR: 303), *C. rectus* (OR: 90), and *T. denticola* (OR: 25); a borderline OR was found for the orange complex (OR: 5.0). The association between *A. viscosus* and PI HAART was statistically significant ($P = 0.03$). In the NNRTI HAART group, lower OR were documented. The highest OR was found for *F. nucleatum* (OR: 56), followed by *E. corrodens* (OR: 25). *P. micra* showed a borderline OR of 5.0 (Table 2).

Discussion

The present investigation found an association between key drugs of antiretroviral therapy, PI and NNRTI, and subgingival bacteria, which, to the best of our knowledge, has not been previously documented. A significant association was found for *A. viscosus* (OR: 303, $P = 0.03$) in the patients on PI HAART. *C. rectus* (OR: 90) and *T. denticola* (OR: 25) also showed high OR for protease inhibitors, whereas *F. nucleatum* (OR: 56) and *E. corrodens* (OR: 25) were more prevalent in NNRTI HAART. The results suggest an influence of PI and NNRTI containing HAART on the prevalence of subgingival bacteria. The OR we found were, to some extent, higher than descriptions of former clinical investigations, which presented OR of 2.6 for periodontal disease progression in patients undergoing zidovudine antiretroviral therapy.[12] The RTI inhibitor zidovudine is still used in contemporary HAART as a so-called backbone drug. The interactions between antiretroviral drugs and periodontal diseases have not yet been elucidated, but clinically, there seems to be more relevant constitution than general immune reconstitution under HAART that normalizes immune response in the HIV-infected patient. In the oral cavity, the mechanisms of HIV-related immune suppression were described as follows: (a) a limited chemotaxis of neutrophilic granulocytes in the initial phase of periodontal disease defense;[18,19] (b) a dysfunction of macrophages with CD4 surface markers in the succeeding phase of phagocyte activity;[20] and (c) a disorganization of gingival B- and T-lymphocyte activity and their humoral products, such as immune globulins.[21] Based on this, all stages of the host-dependent immunological defense against periodontal disease are compromised by HIV infection. It was demonstrated *in vitro* and *in vivo* that PI has a negative effect on the viability and growth of *Candida albicans* (*C. albicans*). Both HIV-1 protease and the virulence factor secreted aspartyl proteinase of *C. albicans* belong to the aspartyl proteinase family, which is suppressed by antiretroviral protease inhibitors. These findings indicate the antifungal activity of PI.[10,22] In a Greek study, a lack of reduction of oral lesions in patients on antiretroviral therapy without any key drug, even though presenting better hematological and viremic data when compared with that of patients on PI HAART, indicated that other factors, besides immune reconstitution, might affect oral lesion reduction in patients undergoing PI HAART.[9] Only one study was found that addressed NNRTI and HIV-related oral lesions.[13] In Aquino-Garcia *et al.*'s[13] study, oral lesions were compared in patients on NNRTI HAART versus PI HAART. The results showed a significantly lower prevalence for HIV-related oral lesions in patients undergoing NNRTI HAART compared to PI HAART. Specifically, lower prevalence was documented for oral candidiasis, necrotizing ulcerative periodontitis, and non-specific oral lesions.[13] In patients

Table 2. Odds ratios for periodontal pathogens associated with PI HAART and NNRTI HAART

Periodontal pathogens and complexes	NNRTI/HAART				PI/HAART				Odds ratio of positive number of patients with PI as reference HAART and NNRTI as HAART at risk	Odds ratio of positive number of patients with NNRTI as reference HAART and PI as HAART at risk	*P*-value
	No. samples	Positively-tested patients	Median SNR	95% CI	No. samples	Positively-tested patients	Median SNR	95% CI			
Aggregatibacter actinomycetemcomitans	24 (n = 6 patients)	1	0.0	0.0, 19.2	16 (n = 4 patients)	1	0.0	0.0; 381.1	0.6	1.7	1.0
Actinomyces viscosus		0	0	0, 0		3	22.7	0.0, 80.3	0.003	303.3	0.03
Tannerella forsythia		5	133.4	9.7, 281.9		3	235.5	0.0, 505.0	1.7	0.6	1.0
Campylobacter rectus		3	8.1	0.0, 29.4		4	35.9	9.2, 56.8	0.01	90.0	0.2
Eikenella corrodens		1	0.0	0.0, 12.1		0	0	0, 0	24.5	0.04	1.0
Fusobacterium nucleatum		6	184.2	40.4, 371.9		3	163.0	0.0, 528.7	55.7	0.01	0.4
Porphyromonas gingivalis		3	7.3	0.0, 152.2		2	47.4	0.0, 556.6	1.0	1.0	1.0
Prevotella intermedia		3	8.4	0.0, 193.8		3	38.5	0.0, 358.8	0.3	3.0	0.6
Parvimonas micros		5	34.8	11.3, 59.2		2	8.0	0.0, 56.1	5.0	0.2	0.5
Treponema denticola		5	0.0	0.0, 248.9		4	190.9	105.4, 320.8	0.04	24.5	1.0
Red complex		2	336.5	84.2, 565.4		2	446.4	0.0, 1326	0.5	2.0	1.0
Orange complex		1	296.4	77.6, 556.6		2	248.7	0.0, 947	0.2	5.0	0.5

CI, confidence interval; HAART, highly-active antiretroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; SNR, signal-to-noise ratio.

with chronic periodontitis, it was evident that HIV-infected patients undergoing HAART had significantly improved periodontal health after scaling and root planing and supportive periodontal therapy. The average pocket reduction was 1.7 mm, demonstrating no significant difference compared with a HIV-seronegative control group.[23] Another study showed that SPT in HIV-infected patients was able to improve gingival health significantly by 50%.[24] These clinical findings demonstrate that HIV-infected patients on HAART could be periodontally treated according to the current treatment concepts. However, it was also noticed that HIV-related oral lesions increased again with the duration of HAART, suggesting that opportunistic HIV-related oral lesions might form part of the clinical picture of immune reconstitution inflammatory syndrome, although that infections were of late onset.[25] While it is advantageous to have first clinical and microbiological data for patients with chronic periodontitis undergoing different HAART regimens, this work has several limitations. The sample of patients was small and included only two commonly-used HAART regimens. Therefore, the risk of bias is substantial. No microbial testing was performed at baseline for comparison after treatment. However, recolonization of the subgingival biofilm in periodontal pockets after scaling and root planing takes up to 60 days to reach pretreatment values.[26] In this respect, the different durations of supportive periodontal therapies documented in the present study would not be expected to be a confounding factor. The patients were also treated according to a standard procedure before microbial testing for clinical standardization of the study population. This could be verified by the intergroup and intragroup statistical comparisons. However, the number of periodontal pathogens in subgingival biofilms far exceeds the 10 tested bacterial species. It has been shown that more than 700 species colonize the oral cavity.[27] Future directions for this particular research collaboration include the verification of the results in a larger cohort, including other HAART regimens, as well as a longitudinal and clinically-accompanied observation of these patients to evaluate clinical relevance. It is of further interest to scrutinize the cellular/molecular effect of antiretroviral drugs on periodontal pathogens, as shown before in *Candida* species.

Conclusion

The results of the present study demonstrated statistical associations between subgingival bacteria and antiretroviral drug therapies. These preliminary results support the generation of a working hypothesis that different antiretroviral therapies might have an influence on subgingival bacteria. Further investigation on the clinical significance and underlying mechanisms are needed to support these findings.

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