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Microbial profiles of patients seeking treatment for periodontitis

Influence of origin, smoking and age?

Key words: periodontitis, microbial profile, epidemiology, *A. actinomycetemcomitans*, *P. gingivalis*

Summary Purpose: We assessed the potential influence of the origin, the smoking status and the age on subgingival microbial profiles of subjects seeking periodontal care in Switzerland today.

Material and Methods: Subgingival samples were obtained from 182 subjects originating from 44 countries (56 native Swiss, 64 other European, 43 African, 19 others), seeking periodontal treatment at the School of Dental Medicine at the University of Geneva. Four periodontal microorganisms were quantified by direct hybridization with specific RNA probes.

Results: *Tannerella forsythia* and *Treponema denticola* were ubiquitous (95.6%, 93.9%), and *Porphyromonas gingivalis* was frequently detected (89%). Counts correlated with the

size of the microbial sample (total load). *Aggregatibacter actinomycetemcomitans* was detected in only 70 (38.4%) subjects. Counts were highly variable and unrelated to total load. Subjects less than 46.8 years old (median age) had a higher risk to be positive than older subjects. Detection frequencies and counts of all four organisms were unrelated to the origin or the smoking status.

Conclusions: Based on a clinical diagnosis of untreated periodontitis, positive outcomes of tests for *T. forsythia*, *T. denticola* and *P. gingivalis* could be predicted with high confidence irrespective of a patient's origin, smoking status or age. Detection of *A. actinomycetemcomitans* was less frequent and depended on the age of the subject.

Introduction

Treatment of periodontal diseases targets a highly diverse microbiota residing on subgingival tooth surfaces (KROES ET AL. 1999, PASTER ET AL. 2001). A few Gram-negative bacterial species are suggested among the numerous recognized taxa to play a significant role in the disease process (SOCRANSKY & HAFFAJEE 1991). Microbiological tests are available to dentists to determine the presence of these organisms. There is an ongoing debate on the utility of such testing in clinical practice. To determine the quality of being of practical use, detailed infor-

mation is needed on how a test or diagnostic algorithm works in a specific setting and what the consequences of a positive or negative test might be. In previous papers our group addressed the questions of whether the presence or absence of periodontal pathogens can distinguish between subjects with chronic or aggressive periodontitis (MOMBELLI ET AL. 2002), and whether microbial testing could indicate success or failure of non-surgical therapy earlier than a clinical assessment at six months (BROCHUT ET AL. 2005). The present report addresses another issue of diagnostic tests: Assays are often judged primarily with respect to sensitivity and specificity to identify a

phenomenon under investigation – high sensitivity is desired in order not to miss any positive cases, whereas high specificity is sought to avoid false positives (YERUSHALMY 1947) – underestimating the importance of the predictive value. The predictive value depends on the prevalence of the condition of interest within the tested population. Thus, the utility of a test to identify a microbial phenomenon (perhaps indicating an advantage for applying a specific antimicrobial therapy) depends on the prevalence of the target microbiota, and may vary in clinically distinct populations.

Similar to other European countries, Switzerland has been subject to important demographic changes in recent years (SWISS FEDERAL DEPARTMENT OF HOME AFFAIRS 2010). Microbial profiles may differ in immigrants and the Swiss population. This is true for medical pathogens, for example *Mycobacterium tuberculosis* (BODENMANN ET AL. 2009), and may be the case for oral microorganisms as well. Somewhat conflicting data on the prevalence of periodontal microorganisms have been reported from different regions of the world, possibly reflecting an influence of local or ethnic risk factors for colonization with putative pathogens. A high prevalence of suspected periodontal pathogens has been reported from certain populations, particularly from developing countries (AL-YAHFOUFI ET AL. 1994, DAHLÉN ET AL. 1989, EISENMANN ET AL. 1983, GMÜR & GUGGENHEIM 1994, MOMBELLI ET AL. 1998). In a study on refugees arriving from non-industrialized countries, even samples from sites with minimal periodontal disease showed a relatively high detection frequency of *Porphyromonas gingivalis* and *Prevotella intermedia* (MCNABB ET AL. 1992).

Detection frequencies may also vary with the smoking status and age. It has been suggested that cigarette smoking increases the risk for subgingival infection with periodontal pathogens (ZAMBON ET AL. 1996). Higher counts of putative periodontal pathogens have been reported from smokers than never-smokers (GOMES ET AL. 2006). Environmental conditions influencing the attachment, growth and metabolism of microorganisms in the oral ecosystem may change with age (MOMBELLI 1998). *P. gingivalis* seems to assume greater importance with increasing age (SAVITT & KENT 1991, TANAKA ET AL. 2002, UMEDA ET AL. 1998). However, these differences may in part be attributed to differences in ecological conditions prevailing in shallow or deep pockets, the latter being more prevalent in the elderly and in smokers. In contrast, *A. actinomycetemcomitans* seems to be related to young subjects with decreasing prevalence and concentration levels in older age groups (SAVITT & KENT 1991, VAN DER WEIJDEN ET AL. 1994).

Classical studies on the effect of various periodontal therapies were conducted to a large part in Caucasians who smoked (... by periodontists who smoked). Today in Europe, an increasing proportion of patients needing periodontal care are non-smokers and come from populations with diverse ethnical backgrounds.

The aim of this analysis was to assess in subjects with untreated periodontitis who seek periodontal treatment at the School of Dental Medicine at the University of Geneva the influence of origin, smoking status and age on the detection of four periodontal microorganisms by direct hybridization with specific RNA probes.

Materials and methods

This paper reports cross-sectional data of subjects investigated in a recruitment campaign for participation in a randomized clinical trial carried out by our service. The protocol was ap-

proved by the appropriate medical ethical committee (Commission Centrale d’Ethique, Hôpitaux Universitaires de Genève) and was authorized by the Swiss Agency for Therapeutic Products (Swissmedic). Research was conducted according to the principles outlined in the Declaration of Helsinki on experimentation involving human subjects.

Subjects

From June 2008 to June 2010, subjects seeking periodontal treatment at the School of Dental Medicine at the University of Geneva were offered to be screened for eligibility to participate in a randomized, placebo-controlled study evaluating the benefit of an adjunctive antibiotic regime. The screening procedure included questions regarding medical and dental health, smoking status (never-smoker, former smoker, <10 or ≥ 10 cigarettes per day) and country of origin, a brief oral clinical exam and sampling of the subgingival microbiota. An informed consent was obtained for all the patients included in this study.

The inclusion criteria were: Age at least 18 years, presence of 12 scorable teeth, not counting the third molars, a clinical diagnosis of chronic or aggressive periodontitis with presence of at least 4 teeth with a probing depth ≥ 5 mm, clinical attachment loss ≥ 2 mm and radiographic evidence of bone loss. Exclusion criteria were systemic illnesses (i.e. diabetes mellitus, cancer, HIV, bone metabolic diseases or disorders that compromise wound healing, radiation or immunosuppressive therapy), pregnancy or lactation, systemic antibiotics taken within the previous 2 months, use of non-steroid anti-inflammatory drugs, and subgingival scaling and root planning or surgical periodontal therapy in the last year.

Microbiological examination

One subgingival microbial sample was obtained in each quadrant from either the deepest pocket or the mesial aspect of the first molar using the paper point method (MOMBELLI ET AL. 1994, MOMBELLI ET AL. 1991). The tooth to be sampled was first isolated with cotton rolls. Supragingival plaque was removed using a cotton pellet. Subgingival samples were then collected with a medium-sized endodontic paper point inserted to the bottom of the pocket and left for 10 s. The samples from the four quadrants were pooled in a transport vial containing 4 M guanidinium thiocyanate 2-mercaptoethanol and sent to the laboratory for analysis.

The samples were analyzed using oligonucleotide probe technology according to standard procedures (DIX ET AL. 1990). They were hybridized to a specific probe for the *ssrRNA* of *A. actinomycetemcomitans*, *P. gingivalis*, *Tannerella forsythia*, *Treponema denticola*, and to a universal bacterial probe (Institut für angewandte Immunologie, Zuchwil, Switzerland). Bacterial counts were calculated by comparison with homologous reference standards and expressed as count $\times 10^6$.

Statistical analysis

Data were entered into a database and were checked for entry errors. Subjects were grouped according to country of origin as follows: Switzerland, other European country, Africa, other. Backward stepwise logistic regression was used for prediction of the probability of detecting the target microorganisms by the predictor variables origin, smoking status, age and bacteriologic sample size (expressed as log total bacterial load determined with the universal probe). The association between the detection and non-detection of different target microorganisms in the same bacteriologic specimen was analyzed with

Fisher's exact Test. Linear regression was used for modeling the relationship between log-transformed counts of the target organisms in positive samples and origin, smoking status, age and log-transformed total loads.

One statistical program package (PASW Statistics 18 for Mac OS X, IBM Corporation, Somers, NY USA) was used for all statistical analyses.

Results

We identified 206 persons with untreated periodontitis that met the inclusion criteria. 182 of these also fulfilled none of the exclusion criteria and accepted to be examined microbiologically. The 182 subjects originated from 44 countries: 56 (31%) persons were native Swiss, 64 (35%) came from another European country, 43 (24%) originated from Africa, and 19 (10%) came from elsewhere. The mean age was 47.9 years

(range 18–75 years). 76 (42%) subjects were female, 77 (42%) were smokers (54% of the native Swiss, 49% of the other Europeans, 30% of the Africans, 16% of the others). Figure 1 displays the age distribution in the four categories of origin. Figure 2 shows the age distribution in the four smoking categories.

Table I shows the frequency of detection of *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* and *T. denticola* by origin. Table II shows the frequency of detection in subjects that never smoked in comparison to those that currently or previously smoked. Table III shows these frequencies in the younger and the older half of the sample (age > or ≤ the median of 46.8 years). As can be seen clearly, *T. forsythia* and *T. denticola* were ubiquitous, with detection frequencies approaching 100%. *P. gingivalis* was detected in 89% of the cases. In contrast, *A. actinomycetemcomitans* was detected in only 38.4% of the subjects.

Backward stepwise logistic regression was used for prediction of the probability of detecting *A. actinomycetemcomitans* or

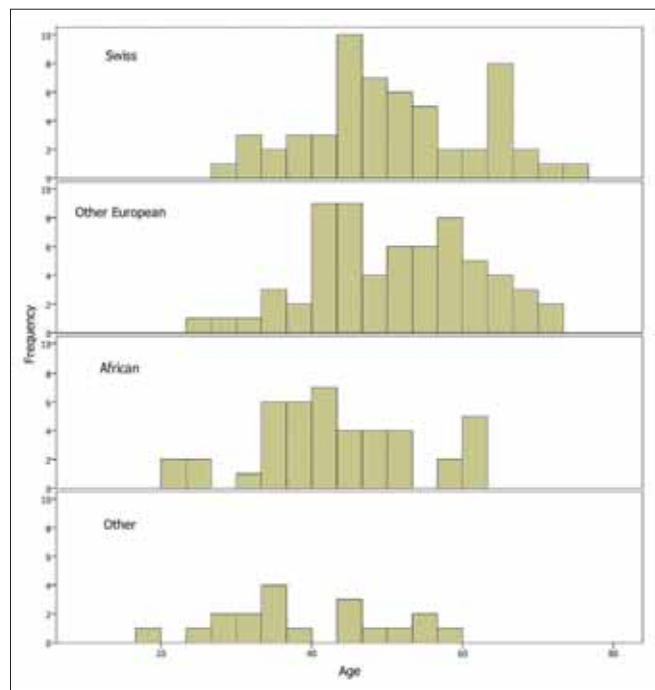


Fig. 1 Age distribution by ethnic group, n=182 subjects

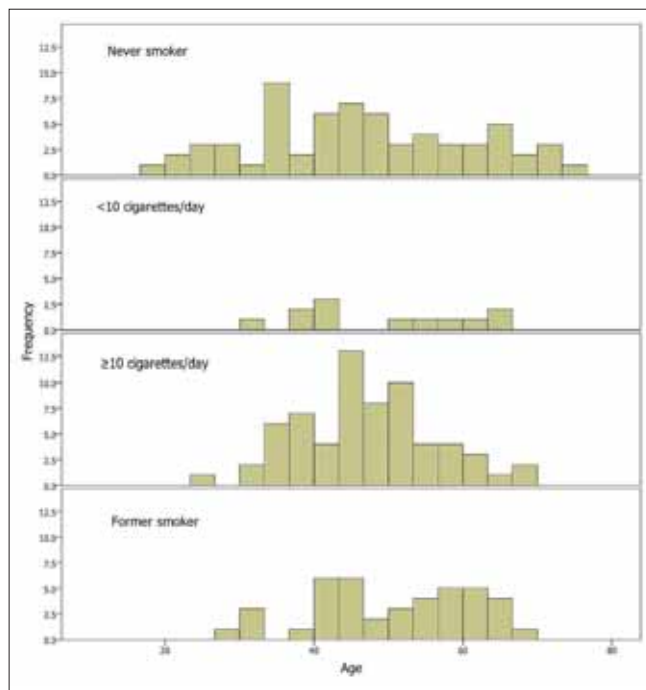


Fig. 2 Age distribution by smoking status, n=182 subjects

Tab.I Frequency of detection and origin					
Origin	N	<i>A. actinomyce-temcomitans</i>	<i>P. gingivalis</i>	<i>T. forsythia</i>	<i>T. denticola</i>
Switzerland	56	23 (41.1%)	51 (91.1%)	56 (100%)	55 (98.2%)
Other European	64	20 (31.3%)	56 (87.5%)	60 (93.8%)	59 (92.2%)
Africa	43	18 (41.9%)	39 (90.7%)	42 (97.7%)	40 (93%)
Other	19	9 (47.4%)	16 (84.2%)	16 (84.2%)	17 (89.5%)
Total	182	70 (38.4%)	162 (89%)	174 (95.6%)	171 (93.9%)

Tab.II Frequency of detection and smoking					
Smoking	N	<i>A. actinomyce-temcomitans</i>	<i>P. gingivalis</i>	<i>T. forsythia</i>	<i>T. denticola</i>
Yes	77	28 (36.4%)	69 (86.9%)	76 (98.7%)	75 (97.4%)
No	105	42 (40%)	93 (88.6%)	98 (93.3%)	96 (91.4%)
Total	182	70 (38.4%)	162 (89%)	174 (95.6%)	171 (93.9%)

Tab. III Frequency of detection and age group

Age group	N	<i>A. actinomycetemcomitans</i>	<i>P. gingivalis</i>	<i>T. forsythia</i>	<i>T. denticola</i>
≤46.8 years	91	41 (45.1%)	39 (42.9%)	85 (93.4%)	89 (97.8%)
>46.8 years	91	29 (31.9%)	28 (30.8%)	89 (97.8%)	82 (90.1%)
Total	182	70 (38.4%)	162 (89%)	174 (95.6%)	171 (93.9%)

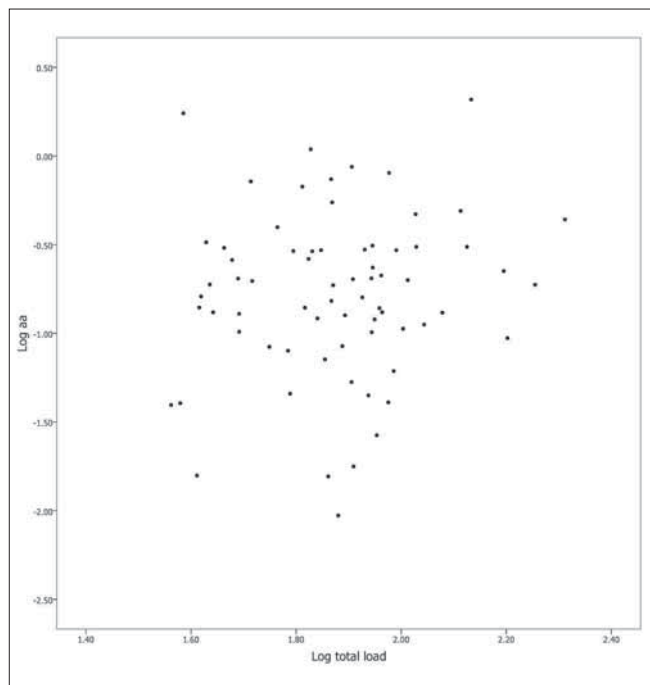
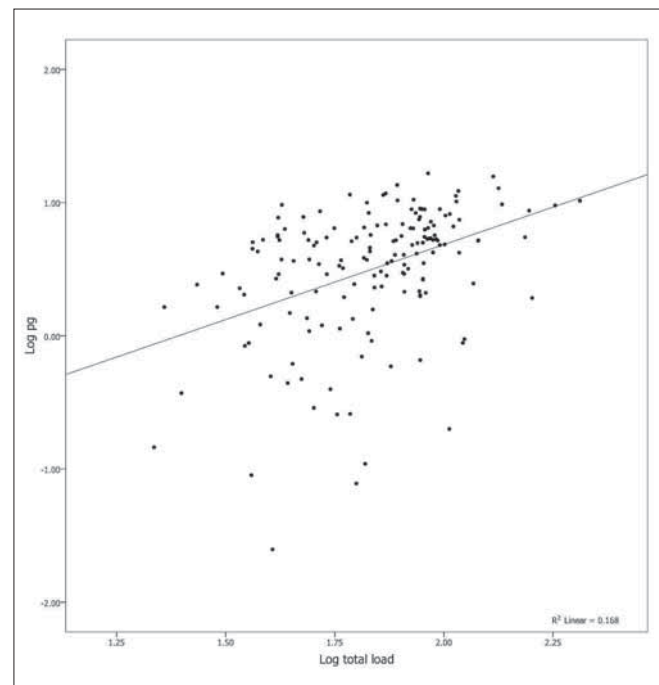
Tab. IV Relationship between detection or non-detection of *A. actinomycetemcomitans* and *P. gingivalis*

		<i>P. gingivalis</i> Negative	Positive	Total
<i>A. actinomycetemcomitans</i>	Negative	17	95	112
	Positive	3	67	70
Total		20	162	182

P. gingivalis using the predictor variables origin, smoking status, age and bacteriologic sample size. The age in years ($p < 0.009$) and the log total bacterial load ($p < 0.001$) were predictors for the detection of *A. actinomycetemcomitans*. Subjects with an age below the median of 46.8 years had a higher risk to be positive than older subjects (odds ratio 2.0, $p < 0.032$). However, the presence or absence of *A. actinomycetemcomitans* was independent of the smoking status (current smoker or past/never-smoker) or the origin (Switzerland, other European country, Africa, other). For detection or non-detection of *P. gingivalis*, backward stepwise logistic regression analysis retained solely the log total bacterial load ($p < 0.003$) as a variable with significant impact. Thus, in the subjects of this study, the detection risk for *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* and *T. denticola* was independent of their origin and smoking status; older subjects were less likely to be *A. actinomycetemcomitans*-positive. The age did not affect the risk of testing positive for the other three target organisms.

Table IV addresses the issue of concurrent detection of *A. actinomycetemcomitans* and *P. gingivalis*. The association between the two tests was significant (Fischer's exact Test $p < 0.017$). In fact, 67 out of 70 samples positive for *A. actinomycetemcomitans* were also positive for *P. gingivalis*. With one exception, all *A. actinomycetemcomitans*-positive persons were also positive for *T. forsythia* and *T. denticola* (a man originating from Haiti, smoking ≥ 10 cigarettes per day, aged 28 years, tested *A. actinomycetemcomitans*-positive but negative for the other three target organisms).

The relationship between the counts of target organisms and total load are shown in graphical form in Figures 3 (*A. actinomycetemcomitans*), 4 (*P. gingivalis*) and 5 (*T. forsythia* plus *T. denticola*). Counts of *P. gingivalis* ($p < 0.001$), and of *T. forsythia* plus *T. denticola* ($p < 0.001$), were significantly correlated in positive samples to the total load. In contrast, the contribution of *A. actinomycetemcomitans* to the total count was highly variable and unrelated to the total load. The counts of all four organisms

Fig. 3 Relationship between log count of *A. actinomycetemcomitans* and log total load (n.s.)Fig. 4 Relationship between log count of *P. gingivalis* and log total load ($p < 0.001$)

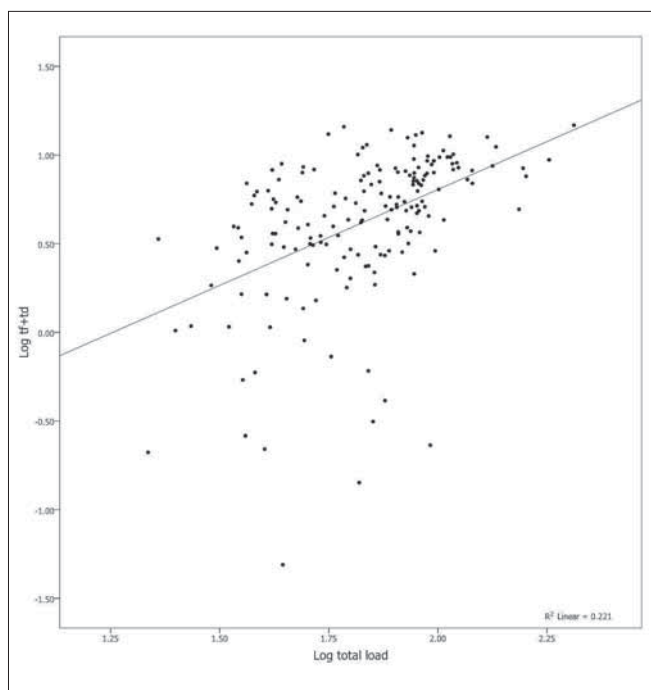


Fig. 5 Relationship between log count of *T. forsythus* plus *T. denticola* and log total load ($p < 0.001$)

in positive samples were unrelated to the origin, smoking status or age of the subject.

Discussion

This study was conducted in the Canton of Geneva, Switzerland, with a population of 458,000 inhabitants, of which 39% do not have Swiss citizenship (OFFICE CANTONAL DE LA STATISTIQUE 2010). The high proportion of immigrants made it possible to study the influence of origin on subgingival microbial profiles. In 182 subjects with a clinical diagnosis of periodontal disease and seeking periodontal treatment, the detection frequencies and counts in positive samples of four periodontal microorganisms were independent of the origin of the person. In a similar study with a comparable sample size ($n = 199$), carried out in the city of Los Angeles, CA, USA (UMEDA ET AL. 1998), “Hispanics” and “Asian-Americans” with moderate or advanced periodontitis had a higher prevalence of *P. gingivalis* than “Caucasians” and “African-Americans”. The first two ethnic groups were not represented in sufficiently high numbers for specific statistical analysis in our cohort (7 individuals from South America, 4 from China).

The detection frequencies of *A. actinomycetemcomitans* and *P. gingivalis* (38% and 89% respectively) were slightly higher than those calculated in a systematic review, where 28% subjects with a clinical diagnosis of chronic periodontitis were *A. actinomycetemcomitans*-positive, and 71% were *P. gingivalis*-positive (MOMBELLI ET AL. 2002). In the present study we did not select subjects with a clinical diagnosis of chronic periodontitis specifically. Our analysis rather concentrated on microbial patterns in subjects aged at least 18 years, with presence of at least 4 teeth with a probing depth ≥ 5 mm, clinical attachment loss ≥ 2 mm and radiographic evidence of bone loss. To what extent current classification systems truly discriminate distinct forms of disease is the matter of an ongoing debate (ARMITAGE ET AL. 2010) that was not the focus of our project. A previous study, carried out in 51 patients with a similar clinical diagno-

sis, and recruited under the same circumstances in our institution, yielded 25% *A. actinomycetemcomitans*- and 83% *P. gingivalis*-positive subjects (CIONCA ET AL. 2010).

As can be seen in Table I, the frequencies of detection of *T. forsythia* and *T. denticola* were very high in all subjects. This reflects results reported in prior literature (for review see SOCRANSKY & HAFFAJEE 2008), indicating that a clinical diagnosis of untreated periodontitis implies the presence of *T. forsythia* and *T. denticola* with high confidence. As there is currently no evidence that *T. forsythia*- or *T. denticola*-negative patients may be at a particular risk, or may benefit exceptionally from a specific form of therapy, testing patients with untreated periodontitis for these two organisms has no practical utility. At best, a positive test may be taken as a confirmation that an appropriate bacteriologic sample was obtained.

67 out of 70 samples positive for *A. actinomycetemcomitans* were also *P. gingivalis*-positive. In other words, if a patient was tested to be *A. actinomycetemcomitans*-positive, one could assume that most probably he was also *P. gingivalis*-positive. We have studied the concurrent detection of *A. actinomycetemcomitans* and *P. gingivalis* previously in 60 young migrant workers in the Province of Guangzhou, People’s Republic of China (MOMBELLI ET AL. 1998). 21 of these subjects were positive for both organisms, whereas 16 were positive for *A. actinomycetemcomitans* but not for *P. gingivalis*. This may suggest that the high predictive value of an *A. actinomycetemcomitans*-positive test to indicate concomitant presence of *P. gingivalis*, *T. forsythia*, and *T. denticola*, may be noted only in subjects diagnosed clinically with periodontitis, reinforcing the need to differentiate the utility of tests in different populations.

In conclusion, based on a clinical diagnosis of untreated periodontitis, positive outcomes of tests for *T. forsythia*, *T. denticola* and *P. gingivalis* could be predicted with high confidence irrespective of a patient’s origin, smoking status or age. Their counts correlated with the size of the microbial sample. Detection of *A. actinomycetemcomitans*, however, was less frequent and was influenced by the age of the subject. Its quantitative contribution to the total microbiota was highly variable.

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Résumé

But: De manière similaire aux autres pays européens, notre pays a été sujet à d’importants changements démographiques ces dernières années. Il a été montré que le profil microbiologique peut différer entre les immigrants et la population résidente. De plus, les fréquences de détection pourraient également varier avec l’âge et la consommation de tabac. Le but de cette étude cross-sectionnelle était d’évaluer l’influence de l’origine, du tabagisme et de l’âge sur le profil microbiologique sous-gingival de sujets avec une parodontite non traitée et consultant de nos jours à Genève.

Matériel et méthode: La détection de quatre pathogènes parodontaux a été investiguée entre juin 2008 et juin 2010 chez 182 sujets souffrant d’une parodontite non traitée et consultant à l’école de Médecine dentaire de Genève. Des échantillons microbiologiques sous-gingivaux ont été prélevés dans les quatre poches les plus profondes ou les faces mésiales des premières

molaires de chaque quadrant. Quatre micro-organismes parodontaux (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia* et *Treponema denticola*) ont été quantifiés par hybridation directe avec des sondes spécifiques à l'ARN ribosomal bactérien. Les données épidémiologiques concernant l'âge, les habitudes de tabagisme et l'origine des sujets étaient collectées à l'aide d'un formulaire.

Résultats: Les 182 sujets inclus étaient originaires de 44 pays différents (56 [31%] Suisses, 64 [35%] en provenance d'autres pays européens, 43 [24%] Africains et 19 [10%] autres). L'âge moyen était de 47,9 ans (entre 18 et 75 ans). 76 (42%) étaient des femmes, et 77 (42%) patients étaient des fumeurs. Concernant la microbiologie, *T. forsythia* et *T. denticola* étaient détectés dans la quasi totalité des échantillons (95,6% et 93,9%). La détection de *P. gingivalis* était moins fréquente (89%). La quantité de ces trois bactéries étaient en corrélation avec la taille totale de l'échantillon microbiologique. *A. actinomycetemcomitans* n'a été détecté que chez 70 (38,4%) sujets. Les quantités de ce micro-organisme dans les échantillons étaient très variables et ne montraient pas de relation avec la charge bactérienne totale. Les sujets âgés de moins de 46,8 ans (médiane) avait un risque plus important d'être positif pour *A. actinomycetemcomitans* que les sujets plus âgés. Les fréquences de détection et les quantités de ces quatre pathogènes parodontaux n'étaient pas liées à l'origine ou aux habitudes de tabagisme des sujets examinés.

Conclusions: Sur la base d'un diagnostic clinique de parodontite non traitée, les résultats positifs des tests pour *T. forsythia*, *T. denticola* et *P. gingivalis* pouvaient être prédits indépendamment de l'origine, des habitudes de tabagisme ou de l'âge des patients. La détection de *A. actinomycetemcomitans* était moins fréquente, et l'âge des sujets avait une influence significative sur la présence de ce micro-organisme dans les échantillons.

Zusammenfassung

Ziel: In der Schweiz haben in den letzten Jahren bedeutende demografische Veränderungen stattgefunden. Wie von allgemeinmedizinisch relevanten Pathogenen bekannt, könnte sich auch das mikrobiologische Profil der subgingivalen Flora von Einwanderern von demjenigen der Einheimischen unterschei-

den. Zudem könnten auch Alter und Tabakkonsum einen Einfluss haben. Das Ziel dieser Studie war es, bei Personen, die heute in Genf mit einer unbehandelten Parodontitis zu einer Konsultation erscheinen, die Einflüsse der Herkunft, der Rauchgewohnheiten und des Alters auf das Profil der subgingivalen Mikroflora zu untersuchen.

Material und Methoden: Bei 182 Personen mit einer nicht behandelten Parodontitis, die die Zahnmedizinische Klinik der Universität Genf zwischen Juni 2008 und Juni 2010 zur Behandlung aufsuchten, wurden subgingivale mikrobiologische Proben entnommen. Die vier Mikroorganismen *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia* und *Treponema denticola* wurden durch direkte Hybridisation mit spezifischen die ribosomale DNA der Bakterien detektierenden Sonden quantifiziert. Epidemiologische Daten bezüglich des Alters, der Herkunft und der Rauchgewohnheiten wurden anhand eines Fragebogens erhoben.

Resultate: Die 182 Personen stammten aus 44 verschiedenen Ländern: 56 (31%) waren Schweizer, 64 (35%) stammten aus anderen europäischen Ländern, 43 (24%) waren afrikanischer Herkunft und 19 (10%) kamen aus anderen Ländern. Das Durchschnittsalter betrug 47,9 Jahre (minimal 18, maximal 75). 76 (42%) Personen waren Frauen und 77 (42%) Raucher. *T. forsythia* (95,6%) und *T. denticola* (93,9%) waren die zwei am häufigsten nachgewiesenen Keime. Ihre jeweilige Quantität war proportional zur Gesamtkeimzahl der mikrobiologischen Probe. *A. actinomycetemcomitans* wurde nur bei 70 (38,4%) Personen detektiert. Die Quantität dieses Keimes war sehr variabel und zeigte keine Beziehung zur Gesamtkeimzahl einer Probe. Personen mit einem Alter unter dem Medianwert (46,8 Jahre) hatten ein höheres Risiko, positiv auf *A. actinomycetemcomitans* getestet zu werden, als ältere Personen. Es bestand kein Zusammenhang zwischen der Herkunft oder dem Tabakkonsum der Probanden und dem Nachweis oder der Quantität der vier untersuchten Keime.

Schlussfolgerungen: Auf der Basis einer klinischen Diagnose einer unbehandelten Parodontitis konnten positive Resultate für *T. forsythia*, *T. denticola* und *P. gingivalis* unabhängig von Herkunft, Rauchgewohnheiten und Alter einer getesteten Person mit hoher Wahrscheinlichkeit vorausgesagt werden. Der Nachweis von *A. actinomycetemcomitans* war weniger häufig und hing signifikant vom Alter der Person ab.

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