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At least three phenotypes exist among periodontitis patients

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Abstract

Aim: To identify phenotypes of periodontitis patients by the use of an unsupervised modelling technique (clustering), based on pre-treatment radiographic and microbiological characteristics.

Materials and methods: This retrospective study included data from 392 untreated periodontitis patients. Co-regularized spectral clustering algorithm was used to cluster the patients. The resulting clusters were subsequently characterized based on their demographics, radiographic bone loss patterns and microbial data.

Results: The vast majority of patients fitted into one of the three main clusters (accuracy 90%). Cluster A (n = 18) was characterized by high prevalence and high proportions of *Aggregatibacter actinomycetemcomitans*, a trend for a more localized pattern of alveolar bone loss and young individuals. Clusters B (n = 200) and C (n = 135) differed clearly in disease severity patterns and smoking habits, but not in microbiological characteristics.

Conclusion: On the basis of alveolar bone loss patterns and microbiological data, untreated periodontitis patients can be clustered into at least three phenotypes. These results should be validated in other cohorts, and the clinical utility needs to be explored on the basis of periodontal treatment outcomes and/or disease progression.

KEYWORDS

bone loss, microbiological data, modelling, periodontitis, phenotypes, radiographic data

1 | INTRODUCTION

Periodontitis is a multicausal inflammatory disease resulting in loss of periodontal connective tissues and alveolar bone support around the teeth. The subgingival dysbiotic microbiome combined with unfavourable genetic and lifestyle factors contributes to the development and progression of the disease (Loos, Papantonopoulos, Jepsen, & Laine, 2015; Lopez, Hujoel, & Belibasakis, 2015; Pihlström, Michalowic, & Johnson, 2005).

Several efforts have been made since the early years to describe and classify periodontal diseases (Armitage, 2002; Van der Velden, 2005). Traditionally, classification systems of periodontal diseases have been mainly based on clinical characteristics (Van der Velden, 2005). The age of disease onset as a discriminatory factor between individuals was introduced in 1969 (Butler, 1969). During the following years (American Academy of Periodontolog, 1989; Attström & van der Velden, 1994; Johnson et al., 1988; Van der Velden, 2005), all the proposed classification systems included the age of disease onset as a criterion for disease classification, until 1999, when Armitage introduced the terms aggressive and chronic periodontitis (Armitage, 1999). These new definitions were based on the assumption that rapid or slow progression of the disease can be present at any age. Van der Velden raised the issue of the clinical applicability of the definitions of chronic and aggressive periodontitis and proposed another, descriptive, classification system which was aimed to be simpler for use in periodontal practice (Van der Velden, 2000).

Armitage referring to his 1999 classification system was also critical on the system and stated: *"It would seem that a more mechanistic or etiological classification could be devised. Why could modern classifications of periodontal diseases not be based on the microbiological features of these infections, or on the genetic factors that seem to control the clinical expression of these diseases?"* (Armitage, 2002).

Recently, a classification based on pathobiology of periodontitis was proposed (Kebschull et al., 2014). In this study, periodontitis patients could be grouped in two biotypes with distinct clinical and microbiological characteristics on the basis of the genetic expression of biomarkers. Interestingly, these biotypes did for the most part not align with the definitions of chronic and aggressive periodontitis.

Data mining and modelling are tools for discovering patterns in complex datasets. Modelling tasks can be performed in a "supervised" and an "unsupervised" manner. One example of supervised modelling is the process of assigning data points to groups/classes and this is known as classification; in supervised modelling, the class label and the number of classes are predefined (Bishop, 2006; Hastie, Tibshirani, & Friedman, 2009). An example of unsupervised modelling is the process of assigning unlabeled data points to groups/clusters using similarity measures, and this process is known as clustering (Bishop, 2006; Hastie et al., 2009). Clustering is the task of grouping patients in such a way that the patients who belong in the same group (cluster) are more similar to each other than to those in other groups (clusters); unlike in classification procedures, the groups are not defined a priori.

We hypothesize that there are different phenotypes among periodontitis patients that can be distinguished based on radiographic alveolar bone loss patterns and microbiological characteristics of the subgingival biofilm. The aim of this study was to cluster periodontitis patients on basis of pre-treatment radiographic alveolar bone loss and microbiological data in order to discover various periodontitis phenotypes and subsequently define their characteristics.

Clinical Relevance

Scientific rationale: To investigate phenotypic heterogeneity among periodontitis patients.

Principal findings: Based on bone loss patterns and microbiological data, the majority of periodontitis patients can be grouped in three clusters. Cluster A consisted of young individuals with a trend for localized bone loss patterns and subgingival *A. actinomycetemcomitans*, while clusters B and C were mainly separated based on disease severity.

Practical implications: These results indicate that there are at least three phenotypes among periodontitis patients. In future, on basis of this type of algorithms, tools may be developed for clinical use and form a basis for disease classification and treatment planning.

2 | MATERIALS AND METHODS

2.1 | Study population

This retrospective study included data from patients referred to the postgraduate clinic of the Department of Periodontology at the Academic Center for Dentistry Amsterdam (ACTA) between 1998 and 2006. Demographic, radiographic and microbiological data of the patients at intake were entered into a secured database and were fully anonymized before any further procedures. This study was conducted in accordance with the principles of the Declaration of Helsinki. The local ethics committee approved the protocol of the current study. The paper adhered to the STROBE guidelines.

All consecutive newly referred periodontitis patients diagnosed by postgraduate students were entered in the study. After exclusion of the patients with missing demographic, microbiological or complete radiographic data, the final cohort for mathematical modelling was formed. Periodontitis was defined as the presence of proximal attachment loss of ≥3 mm in ≥2 non-adjacent teeth (Tonetti & Claffey, 2005).

As a standardized procedure, every new patient underwent microbiological subgingival sampling before periodontal therapy. Sampling was performed by trained and supervised postgraduate students (n = 24). Furthermore, the demographic information of the patients was recorded.

The demographic data collected at the intake were as follows: gender, age, smoking and medical history. Smoking status was defined as current (including former smokers who stopped <1 year ago), former (stopped smoking ≥1 year ago) or never. The medical history was classified according to the American Society of Anesthesiologists Physical Status Classification System (ASA-score). The self-reported diabetes was additionally recorded.

2.2 | Clinical procedures

Patients who visited the Department of Periodontology were initially examined at a diagnostic appointment, in which the periodontal diagnosis and the treatment plan were determined. During this appointment, full-mouth periapical radiographs were taken. Subsequently, at the first appointment for periodontal therapy, and before any further procedures, the deepest non-furcated site in each quadrant was selected for microbiological sampling. After isolating the site with cotton rolls, supragingival plaque was carefully removed using a Gracey curette and the site was gently air-dried. Two subsequent sterile paper points were inserted into the bottom of the pocket for 10s. The eight paper points were pooled, transferred to a reduced transport medium (Syed & Loesche, 1972) and kept at +4°C until processing within 24h.

2.3 | Bacterial detection

Anaerobic bacterial culturing and identification was performed according to a previously described protocol (Van Winkelhoff, Loos, Van der Reijden, & Van der Velden, 2002) for seven periodontal pathogens: *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Tannerella forsythia* (Tf), *Parvimonas micra* (Pm), *Fusobacterium nucleatum* (Fn) and *Campylobacter rectus* (Cr). The total colony forming units per ml (total CFU/ml) were additionally calculated. The microbiological data of the sampled sites were averaged at the patient level for all analyses.

2.4 | Evaluation of radiographs

Anonymized full-mouth radiographs of the study cohort were retrospectively evaluated by 21 dentists and periodontists of the Department of Periodontology. All examiners were provided with a modified version of the Schei ruler (Schei, Waerhaug, Lovdal, & Arno, 1959) (Fig. S1) and received written and oral instructions to identify the cemento-enamel junction, the apex and the most apical aspect of the alveolar bone adjacent to the root as described before (Teeuw et al., 2009). For each patient, missing teeth were noted and all present teeth were scored for the alveolar bone level (no bone loss, bone loss ≤30%, bone loss >30%–≤50% and bone loss >50%) and the presence of angular bony defects. The bone level was determined in the approximal site with the most severe bone loss. The examiners were asked to fill the information on pre-printed forms for each study subject.

2.5 | Clustering and statistical analyses

For the clustering procedure, the microbiological data (seven periodontal pathogens and the total CFU/ml), and the radiographic data (number of teeth present, number of teeth without bone loss, number of teeth with bone loss: ≤30%, >30%–≤50%, >50%, and number of teeth with angular defects) were included. Subsequently, the dataset in all 14 parameters was normalized (zero mean unit variance) (Hastie et al., 2009). The co-regularized spectral clustering algorithm (Biesbroek et al., 2014; Tsivtsivadze et al., 2013) was used to identify the groups/clusters in our data. Each individual patient presents unique characteristics and the clustering groups patients on basis of their similarity to find hidden information or patterns in the dataset. Specifically, each patient gets a probability of belonging to one or more clusters. The highest probability defines in which cluster the patient belongs. For cluster definition and subsequent description, a 0.65 probability threshold of belonging to one cluster was chosen. Thus, the accuracy of the probabilistic cluster assignment was calculated as the percentage of the individuals who were assigned to one specific cluster with a probability of ≥0.65. The method stems from a recently proposed class of spectral analysis algorithms that have been reported to perform superiorly over standard techniques (e.g. k-means, hierarchical clustering, etc.) in accuracy and stability. The algorithm is related to consensus techniques which aim to combine multiple clustering hypotheses for increased accuracy. Furthermore, the algorithm allows identification without supervision, of the optimal number of clusters via construction of co-occurrence matrices and probabilistic cluster assignments (Cornelisse et al., 2012).

The overall comparisons between clusters were performed with Kruskal–Wallis tests, and these were considered statistically significant if $p < .05$. Mann–Whitney testing was applied to define in which combination of clusters the significance emerged; p-values were corrected for multiple comparisons (Bonferroni's correction, $p < .016$). For the comparison of the smoking habits, gender, ASA-score, self-reported diabetes and the percentage of subjects Aa and Pg positive, the chi-square and the Fisher's exact tests were performed.

The co-regularized clustering algorithm was implemented with Python 2.7 (Python Software Foundation, Beaverton, Oregon, USA) and MATLAB R2012b (Math Works, MA, USA). The other statistical analyses were conducted with the SPSS statistical software package (IBM, v.21, Armonk, NY, USA).

3 | RESULTS

3.1 | Study population

A total of 800 consecutive newly referred periodontitis patients were initially diagnosed. After exclusion of the patients with incomplete radiographic or microbiological data ($n = 408$), the final cohort for subsequent analysis consisted of 392 individuals. The demographic-background information and the radiographic and microbiological data of the cohort are summarized in the Tables S1–S3. The mean (median) age of the cohort was 42.2 (43.0) years (range 13–77 years) and 56% were females. Within this population, 41% were current smokers, 31% non-smokers and 28% were former smokers. The majority of the individuals belonged in the ASA1 and ASA2 categories, and the self-reported diabetes of the cohort was 4% (Table S1). The mean (median) number of teeth present was 27.1 (28.0). The mean (median) percentage of teeth with ≤30% bone loss was 42.7% (44.8) while for the categories of bone loss >30%—≤50% and >50% the mean (median) percentages were 27.2% (25.0) and 16.4% (12.5), respectively (Table S2). Applying the classification scheme of Van der Velden (2000), twenty individuals (5%) were classified as juvenile periodontitis (age range 13–20 years), 84 subjects (21%) were classified as post-adolescent periodontitis (age range 21–35 years) and the rest of the cohort was labelled adult periodontitis (Table S2). Table S3 shows the microbiological data of the cohort; on average per patient, the total CFU/ml was 1.8×10^8. Of all patients, 36% were Aa positive, 60% were Pg positive, 19% were Cr positive and >70% were positive for each of the other studied bacterial species. For Aa and Pg, the mean (median) proportions were 2.5% (0.0) and 21.0% (8.0) respectively.

3.2 | Construction of clusters

Unsupervised spectral clustering revealed three groups of patients (Figure 1) as shown in the consensus matrix of co-regularized clustering (Figure 1a) and the probabilistic cluster assignment (Figure 1b). As observed, some patients presented probabilities of belonging in more than one cluster (overlap of the clusters). On the basis of the probability threshold of ≥0.65, the individual patients belonged to one specific cluster, either in cluster A ($n = 18$ [5%]), cluster B ($n = 200$ [51%]) or cluster C ($n = 135$ [34%]). Notably, some patients ($n = 39$ [10%]) did not reach the probability threshold of belonging in any of the three clusters, and these patients are interspersed between clusters A and B ($n = 11$) or between clusters B and C ($n = 28$) and are annotated by x_1 and x_2, respectively (Figure 1). The accuracy for the clustering was 90%.

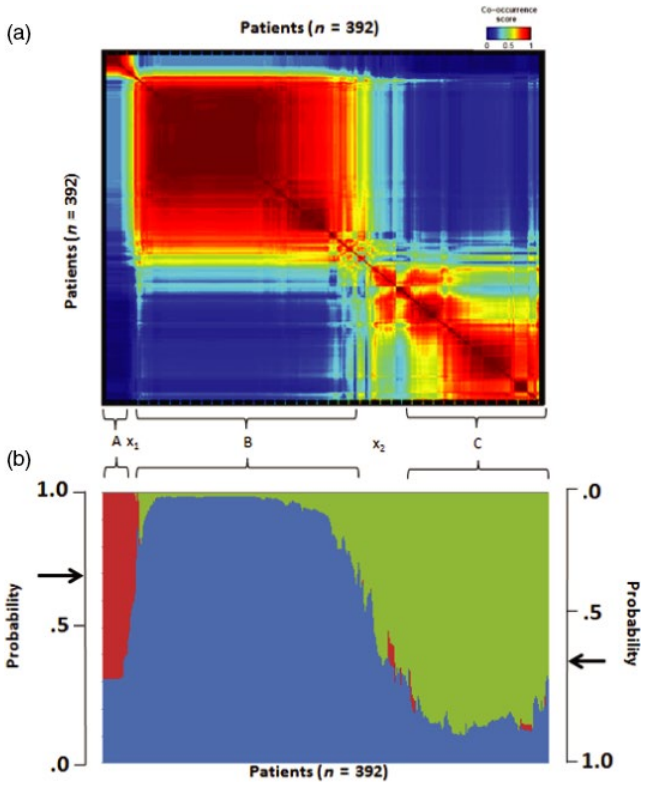

FIGURE 1 (a) Consensus matrix of co-regularized clustering (n = 392): vertical and horizontal axes represent different patients. The co-occurrence score in a pair of patients is depicted with a colour. Red-orange-yellow colours represent high similarity values between two patients. Three clusters (A, B and C) emerged. (b) Probabilistic cluster assignment (n = 392): patients are displayed in the horizontal axis of the figure. Each colour represents the probability of a patient to belong in a specific cluster. Three main colours (clusters) emerged. Overlap of theses colours can be observed, and thus, a patient can be represented in this figure with two or three colours. The latter means that a patient can belong, with a different probability, in more than one cluster. However, within each patient, it is observed that one colour dominates, explaining the highest probability to belong in this cluster. The probability of belonging in a cluster is displayed in the y-axis (0.0–1.0). As observed, the highest probability of 1.0 can be either the higher (left) or the lower value (right) of the y-axis. The threshold of ≥0.65 probability for a patient to belong in one cluster was set in order to assign the patients to the clusters (dominant colour). The separation of clusters A (n = 18, 5%), B (n = 200, 51%) and C (n = 135, 34%) is represented by the brackets. Thirty-nine patients did not reach the probability threshold of belonging in any of the clusters ($X_1 + X_2$ = 39, 10%)

3.3 | Characteristics of the clusters

The demographic and background characteristics of the 3 clusters are presented in Table 1. The cluster A was dominated by young individuals when compared to the other clusters (mean [median] ages in years: cluster A 24.4 [22.5], cluster B 42.8 [42.0] and cluster C 44.2 [44.0], p < .0001) (Table 1). However, periodontitis patients up to the age of 35 years were present in each of the 3 clusters (Figure 2). Significant differences between the clusters were also found for other background factors: (i) the highest percentage of current smokers was observed in cluster C (54%), followed by cluster B (37%), while only 11% of the subjects in cluster A were smokers (p = .0001); (ii) the majority of the individuals in cluster A belonged in the ASA1 category (83%), while approximately half of the individuals in the other two clusters were classified as ASA2 (p = .013); (iii) the highest percentage of self-reported diabetes (7%) was observed in cluster C; however, the latter did not reach a statistically significant difference (p = .083) when compared to the other clusters.

The bone loss characteristics of the clusters are presented in Table 2. Individuals belonging in cluster A had the highest number of teeth present and additionally the highest number of teeth without bone loss indicating a trend for a more localized pattern of the disease (Table 2). Cluster C was characterized by the most severe bone loss, and it was followed by cluster B (Table 2). The mean (median) number of teeth with bone loss >30%–≤50% was 11.3 (11.0) for cluster C, 5.3 (5.0) for cluster B and 3.8 (1.5) for cluster A (p < .0001). Furthermore, the mean (median) number of teeth with bone loss >50% was 8.1 (8.0) for cluster C while it dropped to 2.2 (2.0) and 2.3 (2.0) for clusters B and A, respectively (p < .0001). Lastly, 11 of the 18 patients in cluster A presented localized deep angular defects at the area of the 1st molars and/or incisors (data not shown).

TABLE 1 Demographic and background information of the different clusters (n = 353)

	Cluster A (n = 18)	Cluster B (n = 200)	Cluster C (n = 135)	p-values
Age (years)	24.4 ± 9.2 (22.5)[A,*]	42.8 ± 11.5 (42.0)[a]	44.2 ± 8.6 (44.0)[a]	**<.0001**
Gender				
Females	11 (61%)	118 (59%)	66 (49%)	.16
Males	7 (39%)	82 (41%)	69 (51%)	
Smoking				
No/former	16 (89%)	127 (63%)	62 (46%)	**.0001**
Yes	2 (11%)[b,a]	73 (37%)[b,A]	73 (54%)[B]	
Medical history				
ASA-score[†]				
ASA1	15 (83%)	85 (44%)	48 (37%)	**.013**
ASA2	3 (17%)	101 (53%)	80 (61%)	
ASA3	0 (0%)	5 (3%)	3 (2%)	
Self-reported diabetes	0 (0%)	4 (2%)	9 (7%)	.083

Values represent means ± standard deviations (medians) or numbers (%) of subjects.

*Capital letter as opposed to lowercase letter denotes statistically significant difference between these clusters.

[†]ASA: American Society of Anesthesiologists physical status classification system. The ASA value was not available for 13 patients in the clusters (n = 340).

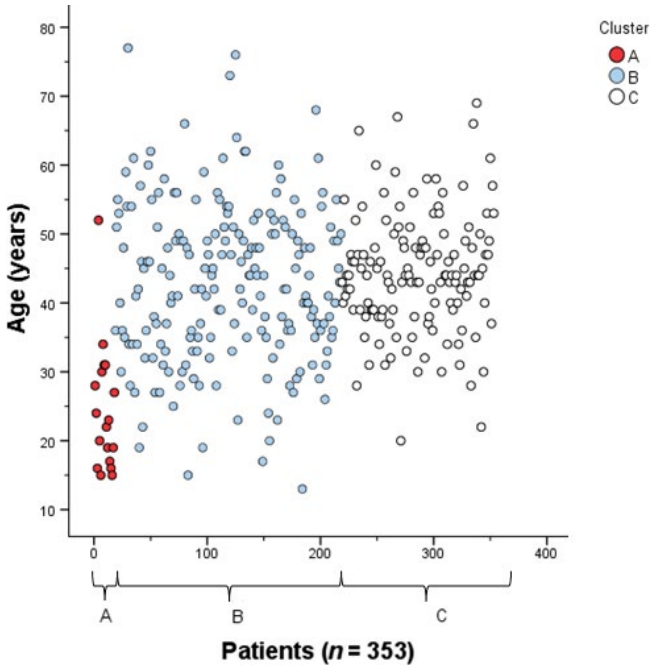

FIGURE 2 Scatter plot of the patients (dots) in the clusters A, B and C per age (n = 353), excluding individuals "x" (n = 39). The horizontal axis represents the overall cohort subjects. The different clusters are illustrated with different colours (A = red, B = blue and C = white). Note that all clusters are represented in the young age categories. Note also that the majority of cluster A (excluding one individual) are below the age of 35 years old

In Table 2, the clusters are compared with the classification scheme of Van der Velden (2000). The majority of the individuals in cluster A were classified as either juvenile periodontitis (44%) or post-adolescent periodontitis (50%), while the majority of the subjects, in both clusters B and C, were classified as adult periodontitis (72% and 85%, respectively). However, all clusters were represented in all categories of the Van der Velden (2000) classification; for example, the percentages of individuals diagnosed as post-adolescent periodontitis were 50%, 25% and 14% for the clusters A, B and C, respectively.

The microbiological characteristics of the clusters are presented in Table 3 and Figure 3. The majority of the individuals in cluster A were Aa positive (89%), while the prevalence dropped to approximately one-third in the other clusters (cluster B 30% and cluster C 35%) (p < .0001) (Figure 3). The prevalence of Pg presented an opposite pattern: in cluster A 22% of the individuals were positive, while this was much higher (62% and 65%) for clusters B and C, respectively (p = .0018). Furthermore, patients in cluster A presented the highest proportion of Aa (mean [median] percentage 35.4 [37.0]) of the cultivable microbiota as compared to both clusters B and C (mean [median] percentages 0.5% [0.0] and 0.7% [0.0] respectively). No differences were observed between the clusters B and C regarding the microbiological culture results (Table 3, Figure 3). Specifically, both clusters in comparison with cluster A presented higher percentage of Pg, Tf, Pm, lower percentage of Aa and higher number of total CFU/ml.

4 | DISCUSSION

In the current retrospective study, we clustered periodontitis patients on the basis of baseline radiographic bone loss patterns and microbiological data in order to investigate possible variation in periodontitis phenotypes. Three clusters of patients emerged. The cluster A was characterized by high percentage and prevalence of Aa and in general a trend for a more localized pattern of the disease. Furthermore, this group consisted of younger individuals as compared to the other two clusters. The clusters B and C did not differ in microbiological characteristics but they differed with respect to disease severity and smoking habits. Both clusters B and C presented higher percentages of Pg, Tf, Pm and total colony forming units as compared to cluster A. Cluster C was characterized by the most severe periodontal destruction and had the highest percentage of current smokers.

Although evidence supports the association of bacteria with disease initiation and progression, to date bacteria have never been included in the proposed classification systems of periodontal diseases. In several longitudinal studies (Fine et al., 2007; Haubek et al., 2008; Timmerman et al., 2000), evidence has shown an association between the presence of Aa and the development of localized aggressive periodontitis or in general the progression of periodontitis. In the current study, we could detect a cluster with high proportion and prevalence of Aa. Individuals belonging to this cluster had additionally a younger age, as compared to the other clusters, a relatively localized pattern of the disease and a high prevalence of localized angular defects at the areas of the 1st molars/incisors. Several clinicians and researchers would assign the diagnosis of localized aggressive periodontitis in the majority of these individuals according the Armitage classification system (Armitage, 1999).

Recent evidence suggests that host genetic background could contribute to a dysbiotic biofilm as it could also play a role in the acquisition, carriership and dominance of bacteria (Nibali, Di Iorio, Onabolu, & Lin, 2016; Offenbacher et al., 2016). Similarly to the current study, Offenbacher et al. (2016) detected an "Aa trait" which was associated with host genetic variants that are specifically involved in the regulation of the neutrophil function. In the same line, Kebschull et al. (2014) clustered periodontitis patients on basis of gingival tissue transcriptomes aiming to develop an alternative classification system on the basis of genetic expression biomarkers. Clustering of these markers resulted in two biotypes of patients with distinct clinical and microbiological phenotypes.

Since 1969 (Butler, 1969), age has been repeatedly included in the disease classification systems until 1999 (Armitage, 1999), when the terms aggressive and chronic periodontitis were proposed. These definitions are based on the assumption that slow or rapid progression of disease can be present at any age. The major criteria for aggressive periodontitis are as follows: patients otherwise clinically healthy, rapid attachment loss and familial aggregation of the disease. However, all these criteria are associated with inherent problems in the clinical practice. Therefore, clinicians often use age in relation to bone or attachment loss, as a surrogate marker for rapid progression and definition of aggressive periodontitis. In the

TABLE 2 Teeth present and bone loss patterns of the different clusters (n = 353)

	Cluster A (n = 18)	Cluster B (n = 200)	Cluster C (n = 135)	p-values
Teeth present	29.6 ± 2.8 (30.5)^{A,*}	27.3 ± 3.0 (28.0)^{a}	26.7 ± 3.0 (27.0)^{a}	**.0001**
Teeth no bone loss	16.2 ± 9.1 (19.5)^{A}	2.6 ± 3.8 (1.0)^{a,B}	0.5 ± 1.4 (0.0)^{a,b}	**<.0001**
Teeth bone loss ≤30%	5.5 ± 3.9 (5.0)^{a}	16.1 ± 4.6 (16.0)^{A}	5.9 ± 4.2 (6.0)^{a}	**<.0001**
Teeth bone loss >30%–≤50%	3.8 ± 5.1 (1.5)^{a,b}	5.3 ± 3.3 (5.0)^{a,B}	11.3 ± 4.7 (11.0)^{A}	**<.0001**
Teeth bone loss >50%	2.3 ± 2.3 (2.0)^{a}	2.2 ± 2.1 (2.0)^{a}	8.1 ± 4.5 (8.0)^{A}	**<.0001**
Teeth with angular defects	2.9 ± 1.4 (3.0)^{a}	3.4 ± 2.1 (3.0)^{a}	6.1 ± 3.3 (6.0)^{A}	**<.0001**
Classification†				
Juvenile periodontitis	8 (44%)	6 (3%)	1 (1%)	
Post-adolescent periodontitis	9 (50%)	49 (25%)	19 (14%)	
Adult periodontitis	1 (6%)	145 (72%)	115 (85%)	

Values represent mean numbers ± standard deviations (medians) of teeth or numbers (percentages) of individuals.
*Capital letter as opposed to lowercase letter denotes statistically significant difference between these clusters.
†Classification of periodontal disease according to Van der Velden (2000).

TABLE 3 Proportions of bacterial species, total CFU and Aa and Pg prevalence on basis of anaerobic culture of the selected sites in the clusters A, B and C

	Cluster A (n = 18)	Cluster B (n = 200)	Cluster C (n = 135)	p-values
Aa	35.4 ± 27.9 (37.0)^{A,*}	0.5 ± 6.1 (0.0)^{a}	0.7 ± 2.6 (0.0)^{a}	**<.0001**
Pg	2.8 ± 6.1 (0.0)^{a}	20.3 ± 23.4 (8.0)^{A}	26.7 ± 27.3 (22.0)^{A}	**.0003**
Pi	1.5 ± 2.8 (0.0)^{a}	4.8 ± 8.0 (1.0)^{A}	3.0 ± 5.0 (0.9)	**.0095**
Tf	2.7 ± 6.4 (0.0)^{a}	8.4 ± 9.3 (5.0)^{A}	8.0 ± 8.3 (6.0)^{A}	**.0001**
Pm	3.8 ± 5.1 (1.0)^{a}	6.4 ± 6.3 (4.0)^{A}	7.9 ± 9.6 (5.0)^{A}	**.0381**
Fn	3.8 ± 5.1 (2.5)	5.1 ± 7.3 (3.0)	4.9 ± 6.7 (3.0)	.4438
Cr	1.6 ± 3.7 (0.0)	0.5 ± 1.5 (0.0)	0.3 ± 1.3 (0.0)	.1985
Total	251.0 ± 279.8	1564.6 ± 2031.9	2641.9 ± 3669.6	**<.0001**
CFU/ml × 10^{8}	(145.0)^{a}	(550.0)^{A}	(76.0)^{A}	
Subjects Aa positive	16 (89%)^{A}	61 (30%)^{a}	47 (35%)^{a}	**<.0001**
Subjects Pg positive	4 (22%)^{a}	125 (62%)^{A}	88 (65%)^{A}	**.0018**

Aa, *Aggregatibacter actinomycetemcomitans*; CFU, colony forming units; Cr, *Campylobacter rectus*; Fn, *Fusobacterium nucleatum*; Pg, *Porphyromonas gingivalis*; Pi, *Prevotella intermedia*; Pm, *Parvimonas micra*; Tf, *Tannerella forsythia*.
In each patient (n = 353), the deepest non-furcated site per quadrant was sampled and the samples were pooled for further analysis.
Values represent mean percentages ± standard deviations (medians) or numbers (%) of subjects.
*Capital letter as opposed to lowercase letter denotes statistically significant difference between these clusters.

present study, intentionally, clustering was performed on the basis of radiographic bone loss patterns and microbiological data, and independent of age and other demographic/background parameters. Interestingly, the patients in cluster A had a mean age of 24.4 ± 9.2; however, it is important to note that young age individuals (below the age of 35 years, often defined as aggressive periodontitis) were

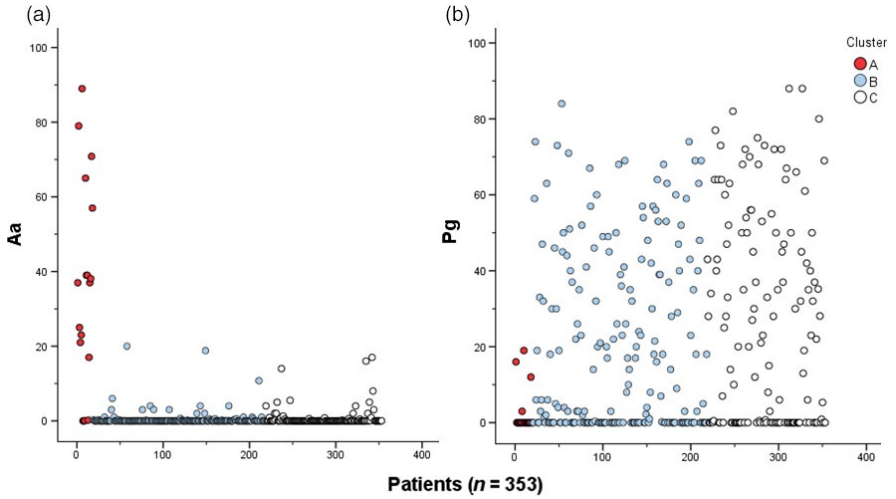

FIGURE 3 Scatter plots of the patients (dots) in the clusters A, B and C. The horizontal axis represents the cohort subjects excluding the individuals "x" (n = 39). The different clusters are illustrated with different colours (A = red, B = blue and C = white). (a) The vertical axis represents the percentage of *Aggregatibacter actinomycetemcomitans* (Aa) per patient. Note the higher percentages of Aa observed in the cluster A. (b) The vertical axis represents the percentage of *Porphyromonas gingivalis* (Pg) per patient. Note that no clear difference between clusters B and C can be observed. Also note the lower percentages of Pg in the cluster A

detected in all clusters. The latter indicates that, although the age at the time of diagnosis is important as it might indicate inter-individual differences with respect to disease susceptibility, it is not the sole factor to determine the classification of the disease, as it has been used until now (American Academy of Periodontology, 1989; Attström & van der Velden, 1994; Johnson et al., 1988; Van der Velden, 2000).

The biological differences between generalized aggressive and generalized chronic periodontitis have been the centre of interest for researchers and clinicians during the past years. In 2010, Armitage, Cullinan, and Seymour (2010) concluded that these diseases did not differ in respect to clinical, microbiological, and histopathological features. However, evidence has shown that localized aggressive periodontitis, presents a distinct clinical and microbiological phenotype (Armitage & Cullinan, 2010; Armitage et al., 2010). In practice, for generalized aggressive periodontitis clinicians use age and familial aggregation in order to discriminate from the generalized chronic periodontitis (Armitage & Cullinan, 2010). Interestingly, we could detect a group (cluster A) with distinct microbiologic characteristics, a relatively localized pattern of the disease and a younger age; while the other two groups did not differ with respect to microbiology or in the age of the individuals.

The clusters B and C differed mainly in disease severity. Cluster C had significantly higher number of teeth with bone loss >30%–≤50% and >50%, and number of teeth with angular defects. The higher prevalence of smoking in cluster C could be one of the factors explaining this difference; however, next to that, other factors such as undetected microbial species, unfavourable lifestyle factors or undiagnosed diabetes could also play a role.

The relatively large sample size, the use of non-invasive markers for cluster definition and the unsupervised statistical approach represent the strengths of the current study. The modelling used in this study is among state-of-the-art for exploratory data analysis and has various computational advantages compared to "standard methods" such as k-means, hierarchical or mixture modelling techniques. The technique does not require any a priori assumptions on the shape or distribution of clusters and allows identification of sub-graphs and manifold structures in the dataset. This feature makes the technique particularly well-suited for clustering high dimensional bone loss and microbiological data. Furthermore, algorithms developed on the basis of radiographic bone levels and microbiological data could be a clinically applicable approach in future for pre-treatment patient's clustering. Nevertheless, clustering techniques have limitations. With the current co-regularized spectral clustering procedure, a patient has his/her probability of belonging to a cluster. The highest probability defines in which cluster the patient belongs. Thus, besides the main, assigned cluster, patients may have also a small probability to belong to another cluster(s). Additionally, some patients did not reach the probability threshold of 0.65 for one of the clusters, and thus, these patients could not be grouped (X patients, n = 39). Larger study populations and inclusion of more/other characteristics may reduce these limitations.

The present study has some other limitations. Firstly, microbiological data are limited to the deepest and non-furcated site per quadrant. However, the same criterion was applied for all patients. Furthermore, a targeted microbiological technique was used. It is recognized that the new open-ended microbiological techniques have revealed in the last years a complex microbiome associated with periodontitis. The balance between the microbiome and the host response may be the determining factor between maintenance of health and transition to disease, and periodontitis is the result of dysbiosis rather than the result of an infection with specific species (Marsh, 1994). Thus, we speculate that the differences observed

between the clusters represent biomarkers of an underlying complex microbiome and/or a possible genetic make-up. Secondly, the radiographic scoring of the alveolar bone destruction was performed, for the purpose of the current study, by several dentists and periodontists of the Department of Periodontology, ACTA, without inter-individual calibration. However, all examiners received clear oral and written instructions on the use of an additional tool, a modified version of the Schei ruler, for the radiographic bone loss scoring. Lastly, the present study describes a specific cohort from the Netherlands (referral university clinic), and the disease characteristics as well as the detection rates of bacteria may vary considerably between geographical regions (Könönen & Gürsoy, 2014); thus, the external validity of the results is unknown. Replication and validation of this study in other populations would strengthen the generalizability of the current findings.

When the resulted clusters were compared with respect to the three level classification system of Van der Velden (2000) (juvenile, post-adolescent and adult periodontitis), differences between clusters for the assigned diagnosis were observed; the clusters did not align with this classification, and all clusters were represented in all categories. We did not compare our findings to the classification system of Armitage (1999) because of the difficulty in assigning the current patients to either aggressive or chronic periodontitis due to the impossibility to apply the criteria, as discussed in the Introduction section. Of special note is the finding that periodontitis patients below the age of 35 years (in many studies often defined as aggressive periodontitis) were present in all clusters (Figure 2). Future work will be the follow-up of the proposed clusters in terms of treatment response and disease progression, and this may give more clinical value to the current clustering results.

In conclusion, on the basis of radiographic alveolar bone loss patterns and microbiological information, periodontitis patients can be clustered in at least three groups with distinct phenotypic characteristics. One group presented high percentage and prevalence of Aa, a trend for a more localized pattern of the disease and younger individuals. The other two groups were mainly differentiated with respect to disease severity and smoking habits.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this study.

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REFERENCES

American Academy of Periodontology. (1989). Proceedings of the World Workshop in Clinical Periodontics. Consensus Report, Discussion Section I. Periodontal diagnosis and diagnostic aids. Princeton, NJ: American Academy of Periodontology.

Armitage, G. C. (1999). Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology*, *4*, 1–6.

Armitage, G. C. (2002). Classifying periodontal diseases -a long- standing dilemma. *Periodontology 2000*, *30*, 9–23.

Armitage, G. C., & Cullinan, M. P. (2010). Comparison of the clinical features of chronic and aggressive periodontitis. *Periodontology 2000*, *53*, 12–27.

Armitage, G. C., Cullinan, M. P., & Seymour, G. J. (2010). Comparative biology of chronic and aggressive periodontitis: Introduction. *Periodontology 2000*, *53*, 7–11.

Attström, R., & van der Velden, U. (1994). Consensus session I. In N. P. Lang, & T. Karring (Eds.), *Proceedings of the 1st European Workshop on Periodontology* (pp. 120–126). Berlin: Quintessence Publishing Co..

Biesbroek, G., Tsivtsivadze, E., Sanders, E. A., Montijn, R., Veenhoven, R. H., Keijser, B. J., & Bogaert, D. (2014). Early respiratory microbiota composition determines bacterial succession patterns and respiratory health in children. *American Journal of Respiratory and Critical Care Medicine*, *190*, 1283–1292.

Bishop, C. M. (2006). *Pattern Recognition and Machine Learning (Information Science and Statistics)*, 1st ed.. New York: Springer.

Butler, J. H. (1969). A familial pattern of juvenile periodontitis (periodontosis). *Journal of Periodontology*, *40*, 115–118.

Cornelisse, L. N., Tsivtsivadze, E., Meijer, M., Dijkstra, T. M., Heskes, T., & Verhage, M. (2012). Molecular machines in the synapse: Overlapping protein sets control distinct steps in neurosecretion. *PLoS Computational Biology*, *8*, e1002450.

Fine, D. H., Markowitz, K., Furgang, D., Fairlie, K., Ferrandiz, J., Nasri, C., … Gunsolley, J. (2007). *Aggregatibacter actinomycetemcomitans* and its relationship to initiation of localized aggressive periodontitis: Longitudinal cohort study of initially healthy adolescents. *Journal of Clinical Microbiology*, *45*, 3859–3869.

Hastie, T., Tibshirani, R., & Friedman, J. H. (2009). *The Elements of Statistical Learning: Data mining, inference and prediction*. 2nd edn, New York: Springer.

Haubek, D., Ennibi, O. K., Poulsen, K., Vaeth, M., Poulsen, S., & Kilian, M. (2008). Risk of aggressive periodontitis in adolescent carriers of the JP2 clone of *Aggregatibacter (Actinobacillus) actinomycetemcomitans* in Morocco: A prospective longitudinal cohort study. *Lancet*, *19*, 237–242.

Johnson, N. W., Griffiths, G. S., Wilton, J. M., Maiden, M. F., Curtis, M. A., Gillett, I. R., … Sterne, J. A. (1988). Detection of high-risk groups and individuals for periodontal diseases. Evidence for the existence of high-risk groups and individuals and approaches to their detection. *Journal of Clinical Periodontology*, *15*, 276–282.

Kebschull, M., Demmer, R. T., Grun, B., Guarnieri, P., Pavlidis, P., & Papapanou, P. N. (2014). Gingival tissue transcriptomes identify distinct periodontitis phenotypes. *Journal of Dental Research*, *93*, 459–468.

Könönen, E., & Gürsoy, M. (2014). Subgingival Distribution of Microorganisms. *Current Oral Health Reports*, *1*, 262–271.

Loos, B. G., Papantonopoulos, G., Jepsen, S., & Laine, M. L. (2015). What is the contribution of genetics to periodontal risk? *Dental Clinics of North America*, *59*, 761–780.

Lopez, R., Hujoel, P., & Belibasakis, G. N. (2015). On putative periodontal pathogens: An epidemiological perspective. *Virulence*, *6*, 249–257.

Marsh, P. D. (1994). Microbial ecology of dental plaque and its significance in health and disease. *Advances in Dental Research*, *8*, 263–271.

Nibali, L., Di Iorio, A., Onabolu, O., & Lin, G. H. (2016). Periodontal infectogenomics: Systematic review of associations between host genetic variants and subgingival microbial detection. *Journal of Clinical Periodontology*, *43*, 889–900.

Offenbacher, S., Divaris, K., Barros, S. P., Moss, K. L., Marchesan, J. T., Morelli, T., & Laudes, M. (2016). Genome-wide association study of biologically informed periodontal complex traits offers novel insights into the genetic basis of periodontal disease. *Human Molecular Genetics*, *25*, 2113–2129.

Pihlström, B. L., Michalowic, B. S., & Johnson, N. W. (2005). Periodontal diseases. *Lancet*, *366*, 1809–1820.

Schei, O., Waerhaug, J., Lovdal, A., & Arno, A. (1959). Alveolar bone loss as related to oral hygiene and age. *Journal of Periodontology*, *30*, 7–16.

Syed, S. A., & Loesche, W. J. (1972). Survival of human dental plaque flora in various transport media. *Applied Microbiology*, *24*, 638–644.

Teeuw, W. J., Coelho, L., Silva, A., van der Palen, C. J. N. M., Lessmann, F. G. J. M., van der Velden, U., & Loos, B. G. (2009). Validation of a dental image analyzer tool to measure alveolar bone loss in periodontitis patients. *Journal of Periodontal Research*, *44*, 94–102.

Timmerman, M. F., Van der Weijden, G. A., Abbas, F., Arief, E. M., Armand, S., Winkel, E. G., … Van der Velden, U. (2000). Untreated periodontal disease in Indonesian adolescents. Longitudinal clinical data and prospective clinical and microbiological risk assessment. *Journal of Clinical Periodontology*, *27*, 932–942.

Tonetti, M. S., & Claffey, N. (2005). Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. Group C consensus report of the 5th European Workshop in Periodontology. *Journal of Clinical Periodontology*, *32*, 210–213.

Tsivtsivadze, E., Borgdorff, H., devan Wijgert, J., Schuren, F., Verhelst, R., & Heskes, T. (2013). Neighborhood Co-regularized Multi-view Spectral Clustering of Microbiome Data. *Partially Supervised Learning*, *8183*, 80–90.

Van der Velden, U. (2000). Diagnosis of periodontitis. *Journal of Clinical Periodontology*, *27*, 960–961.

Van der Velden, U. (2005). Purpose and problems of periodontal disease classification. *Periodontology 2000*, *39*, 13–21.

Van Winkelhoff, A. J., Loos, B. G., Van der Reijden, W. A., & Van der Velden, U. (2002). *Porphyromonas gingivalis*, *Bacteroides forsythus* and other putative periodontal pathogens in subjects with and without periodontal destruction. *Journal of Clinical Periodontology*, *29*, 1023–1028.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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