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ORIGINAL ARTICLE

Persistence of *Porphyromonas gingivalis* is a negative predictor in patients with moderate to severe periodontitis after nonsurgical periodontal therapy

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Abstract
Objectives The aim of this study was to evaluate the quality of prediction for stable results after nonsurgical periodontal therapy by several microbiological variables of the subgingival biofilm and biomarkers of gingival crevicular fluid or oral lavage.
Material and methods Forty-six individuals with moderate or severe chronic periodontitis receiving nonsurgical periodontal therapy were monitored for clinical variables, selected microorganisms, and biomarkers at baseline and 3 and 6 months thereafter. Logistic regression analysis and general linear model (GLM) were applied for analysis of variance and covariance.
Results At 6 months, 20 patients showed a high response (HR) to treatment (at least 60 % of reduction of numbers of sites with PD >4 mm), whereas 26 did not (low response, LR). All clinical variables were significantly improved at 3 and 6 months within each group ($p < 0.001$, each compared with baseline). Modeling the impact of *Porphyromonas gingivalis*, *Treponema denticola*, and median of MMP-8 on to the response to treatment as continuous variables by GLM showed a significant influence of these variables ($p = 0.045$) with the strongest influence of *P. gingivalis* ($p = 0.012$) followed by *T. denticola* ($p = 0.045$) and no association with MMP-8 ($p = 0.982$). Samples tested positively for *P. gingivalis* decreased only in HR (3 months: $p = 0.003$; 6 months: $p = 0.002$). Calprotectin levels in GCF were lower in the HR group compared with the LR group at 3 months ($p = 0.008$) and at 6 months ($p = 0.018$).
Conclusion Persistence of *P. gingivalis* combined with a high GCF level of calprotectin may have a negative predictive value on response to periodontal therapy.
Clinical relevance Microbiological diagnostics for *P. gingivalis* before and 3 months after SRP may have a predictive value on response to periodontal therapy. The combination with MMP-8 in oral lavage or preferably calprotectin in GCF might give additional information.

Keywords Chronic periodontitis · Response to treatment · Biomarkers · Microbiological diagnostics · *Porphyromonas gingivalis*

Introduction

Periodontal destruction is a result of breakdown of the host-microbial homoeostasis [1]. The microbiota of the oral cavity is one of the most diverse microbial community in the human body [2]. The composition of the supra- and subgingival biofilm that is different from the salivary and soft tissue cluster has an important impact on periodontal health or disease [3]. About 500 different species could be differentiated in the subgingival biofilm [4]. However, certain bacteria may play a role as keystone pathogens in changing a symbiotic microbiota to a dysbiotic one; in an animal model, *Porphyromonas*

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gingivalis induced a dysbiosis of the gut microbiota and impaired the gut barrier [5]. *P. gingivalis* lives in symbiosis with *Treponema denticola* and other bacteria essential for the progression of chronic periodontitis [6]. Virulence of *Aggregatibacter actinomycetemcomitans* is mostly based on its production of leukotoxin; the species is associated with aggressive periodontitis [7].

The dysbiotic subgingival biofilm triggers the inflammatory and tissue-degrading responses of the host especially in the adjacent tissues [8]. Different cytokines and matrix-metalloproteinases (MMP), like interleukin (IL)-1β, IL-6, or MMP-8, are important players regarding the tissue destruction [9, 10]. Periodontal disease is monitored via clinical diagnostic variables like clinical attachment level (CAL), probing depth (PD), or bleeding on probing (BOP). These methods have a limited accuracy and deficiencies to predict ongoing or future disease activity as well as to determine whether previously diseased sites are still active or arrested [11, 12]. Host-derived mediators like cytokines, enzymes, or MMPs expressed in different fluids in the oral cavity can be utilized as diagnostic markers for periodontitis [13, 14]. Several attempts have been made to use biomarkers or microbiological data of the gingival crevicular fluid or the subgingival biofilm to improve the quality of the prognosis of periodontal disease progression or stability [15–17]. Besides gingival crevicular fluid, saliva [13, 18] and oral rinse [19] are regarded as a potential tool for diagnosis, progression analysis, and prediction of treatment outcomes of periodontitis.

A combined detection of microorganisms and biomarkers in the saliva correlates with activity of periodontal disease [20–22] and has a predictive value both of progression of disease and response to treatment [23].

The aim of the present study was to evaluate the quality of prediction for stable results after nonsurgical periodontal therapy by several microbiological variables of the subgingival biofilm and biomarkers of gingival crevicular fluid and oral lavage, respectively. The working hypothesis was that a level of MMP-8 in mouthwash above the median combined with the presence of *P. gingivalis* and *T. denticola* 3 months after scaling and root planing (SRP) has a predictive value for 6-month success of treatment. The null hypothesis is that the reduction of microorganisms in the subgingival plaque in association with decreased level of biomarkers in oral lavage is without additional benefit.

Material and methods

Study population

Following approval of the study by the Ethics Committee (#AZ133-12-16042012) of the University of Leipzig Medical Faculty, 55 randomly selected patients were screened and asked to participate in the study. Forty-six volunteers (25 female and 21 male) gave written and informed consent to their participation in the clinical study in two private dental practices (K.V. and M.K.). In obtaining the informed consents and in conducting the study, the principles outlined in the Declaration of Helsinki, as revised in 2008, were followed. Only individuals with moderate or severe chronic periodontitis [24] were included in the study. In addition, two other inclusion criteria were as follows: at least 20 teeth in function and 20 sites with PD ≥4 mm. Individuals were excluded if they had taken antibiotics in the 3 months prior to the study or if they had received periodontal treatment during the previous 2 years. Furthermore, pregnancy, nursing, chronic diseases such as diabetes mellitus or rheumatoid arthritis, and the need of periodontal surgery and/or adjunctive antibiotics to SRP were also criteria for exclusion.

All examination and treatment were performed by the same clinicians (K.V. and M.K.). They were unaware both of the microbiological results and of the biomarker analyses. For inter-examiner calibration, repeated measurements of one quadrant in 10 patients were executed resulting in $\kappa = 0.91$.

Therapy and follow-up treatment

After two initial prophylaxis and instruction sessions, the participants received full-mouth SRP in two sessions carried out within 24 h using hand and ultrasonic instruments under local anesthesia with articaine hydrochloride/epinephrine hydrochloride (Ultracain D-S, Sanofi-Aventis, Frankfurt/Main, Germany). All of the patients used a chlorhexidine digluconate mouthrinse (Chlorhexamed forte 0.2 %, GlaxoSmithKline Healthcare, Bühl, Germany) for 1 min twice daily during the first 7 days after SRP. No adjunctive antibiotics were applied. Careful normal oral hygiene with toothbrush and interdental brushes was performed.

Three and 6 months after SRP, careful supportive periodontal therapy was performed by the same experienced clinicians, and BOP-positive sites were re-instrumented. During the appointment of supportive periodontal care, re-motivation and re-instruction have been performed.

Clinical variables and sampling procedures

Clinical variables were recorded at three appointments: before SRP (baseline), after 3 months, and 6 months. At the same times, biological materials were also sampled. Oral lavage was performed according to a recently described protocol [19]. One milliliter of sterile tap water was applied to the oral cavity. Patients were asked to rinse intensively their teeth for 60 s. Thereafter, suspension was aspirated and given into a tube containing proteinase inhibitors (Sigma-Aldrich, St. Louis, MO, USA). In the following, samples of the subgingival biofilm and gingival crevicular fluid (GCF) were

taken from the deepest site per quadrant. For sampling GCF, sterile paper strips (Periopaper; Oraflow Inc., Smithtown, New York, USA) were placed at the entrance of the periodontal pocket for 30 s. This intracrevicular superficial method [25] ensures that the subgingival biofilm in the pocket is not destroyed. The strips were pooled and placed into a tube containing proteinase inhibitors. After 1 min, endodontic paper points (ISO 60, Roeko GmbH, Langenau, Germany) were inserted into the pocket until resistance was felt and were left in place for 30 s. All samples were stored at −20 °C immediately after collection and transferred to the laboratory on dry ice within 1 month where the plaque samples were again stored at −20 °C, and the oral lavage and GCF samples at −80 °C until analysis.

The clinical variables PD, CAL, and BOP of all teeth were determined in a six-point measurement per tooth (mesiobuccal, buccal, distobuccal, mesiooral, oral, and distooral). The assessment was performed with a manual periodontal probe (PCP-UNC 15, Hu-Friedy Manufacturing Co., Chicago, IL, USA) using a pressure of about 0.25 N. The interproximal plaque index (API) [26] was used as a measure for oral hygiene.

Biochemical and microbiological analysis

Before analyzing, GCF samples were eluted at 4 °C overnight into a 750-μl phosphate-buffered saline. From the eluates and oral lavages, the levels of interleukin (IL)-1β, matrix-metalloprotease (MMP)-8, MMP-1, and tissue inhibitor of matrix-metalloprotease (TIMP)-1 were determined by using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Europe Ltd., Abingdon, UK) and calprotectin by using commercially available ELISA kit (My Biosource, San Diego, CA, USA) according to the manufacturer's instructions. The detection levels of the kits were 0.5 pg/ml for calprotectin and TIMP-1, 2 pg/ml for IL-1β and MMP-1, and 100 pg/ml for MMP-8.

Microbiological analysis was made as described recently [27]. In short, DNA was extracted by using Chelex method [28]. In the following, the microIDent®plus11 test (Hain Lifescience, Nehren, Germany) was used according to the manufacturer's description. The test is able to identify 11 periodontopathogenic bacterial species after two PCR runs and a subsequent reverse hybridization. Intensity of staining of the strips was used for semi-quantification. Two qualified investigators determined independently the score (0 = no band (negative), 1 = weak band (low load), 2 = clear band (moderate load), 3 = strong band (high load), 4 = very strong band (very high load)). Both the reference bands and the hybridization controls were used for adjustment. The sensitivity for the test is 10^3 for *A. actinomycetemcomitans* and 10^4 for the other bacteria. In addition, the counts of *Filifactor alocis* were determined by using real-time PCR. The quantitative results were categorized accordingly: 10^4/sample = 1, 10^5/sample = 2, and $\geq 10^6$/sample = 3.

Data analysis

Power analysis based on a comparison between patients with stable conditions and disease progression [23] revealed 45 participants to detect a significant difference ($p \leq 0.05$) with a test power of 90 %. This study was a longitudinal observational study. At the end, the study population was grouped in a group that highly responded to treatment (HR) and a group with low response (LR). High response to therapy was defined as a reduction of sites with PD >4 mm of minimum 60 % at 6 months, low response to all the others [29, 30]. The primary outcome was the presence of MMP-8 in oral lavage above the median combined with a positive result (detection by the used microbiological kit) both for *P. gingivalis* und *T. denticola* 3 months after SRP.

After the descriptive statistics of all included participants, a logistic regression analysis to model presence of HR (60 % reduction of PD ≥4 mm) against sum of presence of *P. gingivalis* and *T. denticola* and presence of MMP-8 was performed. In further analyses, the influence of the different variables was checked, and those were compared between the groups. Quantitative variables were analyzed using Wilcoxon-matched pair test for paired variables (time dependencies) and Mann-Whitney *U* test (to compare HR vs LR). McNemar (timepoint) and Fisher s exact tests (groups) were executed to compare categorical and dichotomized variables. Spearman correlation associated biomarker results of the oral lavage with those of GCF.

General linear models (GLMs) for analysis of variance and covariance, as well as logistic regression for analyzing the influence of variables that determine treatment outcome, were applied. The level of significance was set to 5 %. Software SAS®9.4 [31] was used.

Results

Study population and clinical variables

The study was performed in 2013 and 2014; the last patient left the study in January 2015. Figure 1 presents the study flow adapted to Moher et al. [32]. Fifty-five patients were assessed for eligibility, and nine patients were excluded. There was no drop out; however, in one patient, biological samples taken at 3 months were not suitable for analysis due to a technical error. Finally, data from 46 patients (21 male, 25 female; mean age 55 years, range 33–77 years) were evaluated. The demographic data of these 46 patients are summarized in Table 1. At 6 months, 20 patients showed a high response to treatment (at

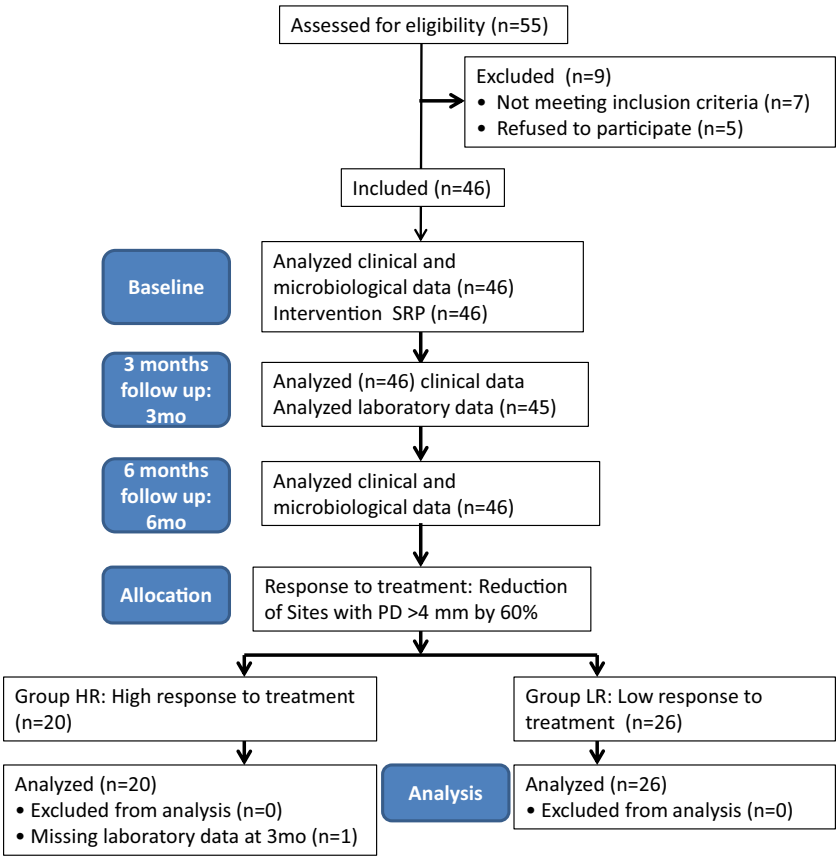

Fig. 1 Flowchart (adapted to Moher et al.) [32] of the study analyzing biomarkers and microbial predictors in patients with chronic periodontitis after nonsurgical periodontal therapy

least 60 % of reduction of numbers of sites with PD >4 mm), whereas 26 did not (Fig. 1).

The age (HR: 55.85 ± 7.44; LR: 55.07 ± 11.40; $p = 0.842$) and the gender distribution (HR: 10 male, 10 female; LR: 11 male, 15 female; $p = 0.766$) of the patients were similar in both groups. Eleven of the 20 patients in the HR group and 8 of the 25 patients in the LR group were smokers ($p = 0.135$). Changes of the clinical data (medians as well as minimum and maximum) observed during the study are presented in Table 2. All clinical variables (mean PD, number of sites with PD ≥4 mm, mean CAL, BOP; API) were significantly improved at 3 and 6 months within each group ($p < 0.001$ each compared with baseline). No significant difference was found for any of the analyzed clinical variables at baseline. Number of sites with PD >4 mm showed a trend to less respective sites in the HR group compared with LR group ($p = 0.053$). BOP and API were never significantly different between the groups at any time. At 3 and 6 months, significant differences ($p < 0.01$) were found for mean PD, number of sites with PD >4 mm, and mean CAL; at 3 months, there was a trend for higher BOP in the LR group ($p = 0.099$).

Table 1 Study population and clinical variables at baseline

Gender (male/female)	21/25
Age (mean ± SD)	55.41 ± 9.79
Smoker (yes/no)	19/27
PD (mean ± SD)	3.96 ± 0.58
Number of PD >4 mm (mean ± SD)	93.00 ± 25.61
CAL (mean ± SD)	4.90 ± 0.77
BOP (mean ± SD)	46.91 ± 13.99
API (mean ± SD)	52.43 ± 12.79

Bacteria being associated with periodontal disease

Analysis was made for categories as well as for dichotomized data. No additional benefit was seen when analyzing categories (suppl. Table 1). In the following, only dichotomized data are analyzed in detail (Table 2). Comparing *Parvimonas micra*, *Fusobacterium nucleatum/necrophorum*, *Campylobacter rectus*, *Eubacterium nodatum*, *Eikenella corrodens*, and *Capnocytophaga* sp. was without significance (suppl. Tables 1 and 2). At baseline, less samples were tested positively for *A. actinomycetemcomitans* in the LR group ($p = 0.047$); this number increased at 3 months in that group ($p = 0.046$). Decrease of samples tested positively for selected bacteria was only seen in the group with high response to treatment. Less positive samples were found for *P. gingivalis* ($p = 0.003$), *P. intermedia* ($p = 0.046$), and *F. alocis* ($p = 0.025$) at 3 months and for *P. gingivalis* ($p = 0.002$), *T. forsythia* ($p = 0.009$),

Table 2 Clinical data at baseline, 3 months, and 6 months after SRP related to response to treatment (defined by 60 % reduction of number of sites >4 mm at 6 months)

	HR group (high response, $n = 20$)		LR group LR (low response, $n = 26$)		HR vs LR (p)
	Median (min, max)	vs baseline (p)	Median (min, max)	vs baseline (p)	
Mean PD (mm)					
Baseline	3.78 (3.12, 4.71)		3.95 (2.97, 5.25)		0.184
3 months	2.99 (2.37, 3.46)	<0.001**	3.61 (2.85, 4.36)	<0.001**	<0.001**
6months	2.78 (2.29, 3.28)	<0.001**	3.38 (2.72, 3.80)	<0.001**	<0.001**
# PD >4 mm					
Baseline	78.0 (41, 135)		101.0 (36, 155)		0.053
3 months	26.5 (12, 72)	<0.001**	85.0 (24, 124)	<0.001**	<0.001**
6 months	17.0 (4, 40)	<0.001**	61.0 (16, 97)	<0.001**	<0.001**
Mean CAL (mm)					
Baseline	4.92 (3.30, 6.57)		4.77 (3.83, 6.66)		0.287
3 months	3.93 (2.62, 5.33)	<0.001**	4.44 (3.46, 5.98)	<0.001**	<0.001**
6 months	3.60 (2.55, 5.14)	<0.001**	4.11 (3.36, 5.68)	<0.001**	<0.001**
BOP (%)					
Baseline	44.2 (30.2, 75.0)		45.8 (23.3, 84.2)		0.313
3 months	30.9 (20.2, 43.3)	<0.001**	34.6 (20.3, 60.0)	<0.001**	0.099
6 months	19.9 (12.0, 32.7)	<0.001**	20.5 (11.2, 39.3)	<0.001**	0.195
API (%)					
Baseline	51.0 (35, 77)		53.0 (35, 82)		0.249
3 months	32.0 (22, 46)	<0.001**	36.0 (21, 58)	<0.001**	0.222
6 months	23.5 (14, 31)	<0.001**	22.5 (18, 38)	<0.001**	0.475

**$p < 0.01$

T. denticola ($p = 0.034$), and *F. alocis* ($p = 0.014$) at 6 months. The analysis of sums of positive results for these six species revealed a significant difference between the groups at baseline with higher sums in the HR group ($p = 0.035$). Only in this group, a decrease was detected at 3 months ($p = 0.004$) and at 6 months ($p = 0.005$).

Biomarkers

TIMP-1 was never detected in GCF. In general, the levels of biomarkers in GCF did not correlate well with those in oral lavage. Only MMP-8 showed at each timepoint a significant association with the highest correlation at baseline ($r = 0.559$; $p < 0.001$). Most differentiating between groups was calprotectin in GCF; the levels were lower in the HR group compared to the LR group at 3 months ($p = 0.008$) and at 6 months ($p = 0.018$). Only two significant intragroup differences were found; the level of IL-1β in oral lavage at 6 months in the LR group ($p = 0.029$) and the level of MMP-1 in GCF at 3 months in the HR group ($p = 0.027$) were lower in comparison with baseline each (Tables 3 and 4).

Variance and covariance

Using the primary outcomes (positive result for *P. gingivalis*, *T. denticola*, and MMP-8 in the oral lavage above the median) resulted in 9 of 26 (34.6 %) patients being positive in the LR group whereas no patient in the HR group was positive ($p = 0.006$). Modeling of these variables on response to treatment as a continuous variable by GLM showed a significant influence of these variables ($p = 0.045$) with the strongest influence of *P. gingivalis* ($p = 0.012$) followed by *T. denticola* ($p = 0.045$) and no association with MMP-8 ($p = 0.982$). However, when neglecting MMP-8, there was no significant difference between the groups anymore (13 (50 %) positive in LR group, 4 (21.1 %) in HR group, $p = 0.065$).

In addition, different other models were applied to check for possible influences by laboratory variables at baseline and 3 months on the success of treatment. Selection was based on the intra- and intergroup differences. Logistic models did not find any significant influences of the major bacteria being associated with periodontitis at baseline on treatment group (HR/LR; model: $p = 0.369$).

In the logistic model at 3 months, the major bacteria as well as the GCF level of calprotectin was included. The model

Table 3 Selected qualitative microbiological data at baseline, 3 months, and 6 months after SRP related to response to treatment (defined by 60 % reduction of number of sites >4 mm at 6 months

	HR group (high response, n =20)			LR group (low response, n = 26)			Gr. HR vs Gr. LR (p)
	n	Positive (%)	vs baseline (p)	n	Positive (%)	vs baseline (p)	
A. actinomycetemcom							
Baseline	20	5 (25.0)		26	1 (3.9)		0.047*
3 months	19	3 (15.8)	0.317	26	4 (15.4)	0.083	0.641
6 months	20	5 (25.0)	1.000	26	5 (19.2)	0.046*	0.543
P. gingivalis							
Baseline	20	15 (75.0)		26	18 (69.2)		0.463
3 months	19	5 (26.3)	0.003**	26	17 (65.4)	0.655	0.010*
6 months	20	5 (25.0)	0.002**	26	15 (57.7)	0.180	0.027*
T. forsythia							
Baseline	20	19 (95.0)		26	23 (88.5)		0.410
3 months	19	15 (79.0)	0.180	26	22 (84.6)	0.655	0.456
6 months	20	13 (65.0)	0.034*	26	23 (88.5)	1.000	0.061
T. denticola							
Baseline	20	18 (90.0)		26	19 (73.1)		0.145
3 months	19	12 (63.2)	0.059	26	16 (61.5)	0.180	0.581
6 months	20	12 (60.0)	0.034*	26	16 (61.5)	0.180	0.577
P. intermedia							
Baseline	20	10 (50.0)		26	9 (34.6)		0.227
3 months	19	5 (26.3)	0.046*	26	5 (19.2)	0.103	0.416
6 months	20	6 (30.0)	0.103	26	4 (15.4)	0.059	0.203
F. alocis							
Baseline	20	19 (95.0)		26	20 (76.9)		0.097
3 months	19	13 (68.4)	0.025*	26	17 (65.4)	0.180	0.385
6 months	20	13 (65.0)	0.014*	26	16 (61.5)	0.103	0.366
Sum of 6 species		Median (min, max)			Median (min, max)		
Baseline	20	5.0 (0,6)		26	4.0 (0,5)		0.035*
3 months	19	2.0 (0,5)	0.004**	26	3.5 (0,5)	0.449	0.466
6 months	20	2.0 (0,6)	0.005**	26	3.0 (0,6)	0.170	0.412

*$p < 0.05$, **$p < 0.01$

itself was not significant ($p = 0.232$); however, a significant influence of *P. gingivalis* ($p = 0.024$; OR 0.057; 95 % confidence 0.005, 0.685) and of GCF calprotectin ($p = 0.021$; OR 0.066; 95 % confidence 0.007, 0.669) on response to treatment group was stated. The followed logistic model including only these two variables became significant ($p = 0.012$) with an influence by *P. gingivalis* ($p = 0.052$; OR 0.253; 95 % confidence 0.063, 1.013) and of GCF calprotectin ($p = 0.036$; OR 0.118; 95 % confidence 0.016, 0.868) on treatment group. GLM procedure modeling of these two variables on response to treatment as a continuous variable resulted in a high significance of the model ($p = 0.001$) with an influence of *P. gingivalis* ($p = 0.026$) and of GCF calprotectin ($p = 0.019$).

In addition, differences of *P. gingivalis* and GCF MMP-1 between baseline and 3 months were modeled. The logistic model was significant on treatment group ($p = 0.036$) with an influence by *P. gingivalis* difference ($p = 0.018$; OR 0.153; 95 % confidence 0.033, 0.719) and of GCF MMP-1 difference ($p = 0.201$; OR 0.957; 95 % confidence 0.893, 1.024).

Discussion

In this study, the predictive value of certain microorganisms in the subgingival biofilm combined with the analysis of selected biomarkers in the GCF and the oral lavage was evaluated. To our best knowledge, a study based on variables of the subgingival compartment and oral lavage has not yet been performed. As the primary outcome variable, the level of MMP-8 in oral lavage

Table 4 Biomarkers in gingival crevicular fluid (GCF) and oral lavage at baseline, 3 months, and 6 months after SRP related to response to treatment (defined by 60 % reduction of number of sites ≥4 mm at 6 months)

	HR group (high response, $n = 20$) vs baseline		LR group (low response, $n = 26$) vs baseline		HR vs LR (p)	Correlation GCF–oral lavage	
	Median (min, max)	(p)	Median (min, max)	(p)		R	p
GCF							
IL-1β (pg/site)							
Baseline	76.61 (25.58, 340.84)	0.263	67.42 (8.77, 281.71)		0.401	0.151	0.327
3 months	55.29 (6.16, 318.76)	0.275	57.20 (9.31, 190.00)	0.245	0.954	0.359	0.015*
6 months	44.56 (7.67, 332.75)		46.30 (9.85, 427.45)	0.053	0.650	−0.030	0.841
MMP-1 (pg/site)							
Baseline	22.33 (9.17, 38.36)		17.33 (9.34, 33.58)		0.100	0.191	0.213
3 months	17.87 (0.23, 46.32)	0.027*	16.54 (7.53, 52.42)	0.378	0.963	0.276	0.066
6 months	20.35 (11.85, 51.64)	0.891	21.47 (10.93, 124.30)	0.105	0.824	0.168	0.271
MMP-8 (pg/site)							
Baseline	2,126 (218, 2,945)		2,234 (1,035, 3,170)		0.605	0.559	<0.001**
3 months	2,091 (218, 2,945)	0.265	2,373 (1,096, 3,222)	0.420	0.228	0.453	0.002**
6 months	2,183 (658, 3,243)	0.734	2,453 (1,020, 3,201)	0.666	0.428	0.318	0.034*
Calprotectin (pg/site)							
Baseline	1.24 (0.79, 3.61)		1.52 (0.55, 2.66)		0.383	0.126	0.413
3 months	1.08 (0.76, 1.87)	0.229	1.40 (0.91, 2.32)	0.512	0.008**	−0.380	0.040*
6 months	1.10 (0.34, 2.37)	0.396	1.45 (0.87, 2.36)	0.179	0.018*	−0.278	0.062
Oral lavage							
IL-1β (pg/ml)							
Baseline	36.65 (0.00, 250.81)		45.49 (0.00, 371.92)		0.530		
3 months	41.41 (0.00, 133.08)	0.468	47.10 (0.00, 145.76)	0.176	0.653		
6 months	33.17 (0.00, 182.78)	0.651	33.10 (0.00, 135.08)	0.029*	0.877		
MMP-1 (pg/ml)							
Baseline	21.19 (0.00, 94.17)		30.94 (0.00, 129.59)		0.391		
3 months	22.91 (4.26, 64.55)	0.182	24.09 (3.03, 60.41)	0.146	0.927		
6 months	29.02 (12.32, 68.79)	0.768	28.70 (8.34, 66.24)	0.471	0.696		
MMP-8 (pg/ml)							
Baseline	2,230 (1,168, 2,977)		2,095 (100, 3,124)		0.758		
3 months	2,146 (537, 2,936)	0.768	2,255 (685, 3,052)	0.744	0.455		
6 months	2,168 (450, 3'194)	0.927	2,012 (105, 3,230)	0.784	1.000		
Calprotectin (pg/ml)							
Baseline	0.40 (0.00, 4.05)		0.25 (0.00, 1.30)		0.261		
3 months	0.32 (0.00, 1.55)	0.074	0.38 (0.00, 1.45)	0.988	0.695		
6 months	0.50 (0.00, 1.75)	0.610	0.27 (0.00, 1.70)	0.761	0.100		

*$p < 0.05$, **$p < 0.01$

above the median combined with a positive result both for *P. gingivalis* und *T. denticola* at 3 months after SRP was chosen. The difference between the high response and the low response groups was significant which was underlined by a GLM model in addition.

In this study cohort, a 60 % reduction of number of sites with PD >4 mm was used as a cutoff to define high and low response to treatment. Persistence of pockets with PD >4 mm is a criterion to classify response to treatment; in mean, a reduction of about 60–>90 % was reported in nonsurgical therapy without antibiotics [29, 30]. Pockets with PD ≤4 mm are related with prognosed periodontal stability as suggested in periodontitis risk analysis systems [33, 34]. An observation time of 6 months with an appointment after 3 months was chosen as clinical variables improve most in the first 4 to 5 months after nonsurgical therapy [35]. Our study using 60 % as a cutoff resulted in 20 patients

with high response and 26 with low response. As no differences in oral hygiene (API) were seen, higher and lower responses to periodontal therapy can be suggested.

Smoking is a well-known risk factor for periodontal disease [36]. It was shown that smokers respond less favorable to nonsurgical periodontal therapy [37, 38]. However, others [39, 40] did not see a clear difference in treatment outcome between smokers and non-smokers like in our study. According to the patients' reports, the heaviest smoker of study participants smoked 20 cigarettes, and the other smokers smoked between 5 and 15 cigarettes per day.

The clinical variables PD and CAL improved statistically significantly more in the HR than in the LR group after 3 and 6 months. There was a tendency to lower BOP in HR group at 3 months and no difference at 6 months. BOP is considered as a predictor of progression in supportive periodontal therapy [41]. Our protocol included the retreatment with SRP of BOP-positive sites at the 3-month appointment which may have influence inflammatory response.

Analyzed in combination with *P. gingivalis* and *T. denticola* (primary outcome) but not as a single variable, the level of MMP-8 in oral lavage was associated with response to treatment. In GCF, the MMP-8 level is reduced after nonsurgical anti-infective therapy [15, 42]. MMP-8 is produced in PMNs and a mediator in initiating responsiveness to lipopolysaccharide [43] and most abundant in GCF [44]. Our used assay determined total MMP-8 not differentiating between active and latent MMP-8. This may weaken differences as periodontitis patients have 80 % active MMP-8, whereas in gingivitis, only 20 % are active [44].

Among the studied biomarkers, calprotectin as a major cytosolic protein of PMNs differentiated most between high and low response to treatment. Already in 2000, it was suggested as a useful marker of periodontal inflammation because of its high correlation with clinical indices and collagenase activity [45]. It is higher in periodontitis subjects [46] and increases during experimental gingivitis in the saliva [47]. Anti-infective periodontal therapy of chronic periodontitis reduces significantly the level of calprotectin in the gingival crevicular fluid [48]. Modeling calprotectin level and *P. gingivalis* presence 3 months after SRP confirmed a significance of both variables. Our results indicate calprotectin in combination with *P. gingivalis* as a predictor for treatment outcome; however, for a routine determination, an easy to use test should be developed.

The major function of TIMPs is the inhibition of MMPs by formation of non-covalent complexes; on the other hand, TIMPs have mitogenic effects, play a role in apoptosis, and may even activate MMPs [49]. Among the family of TIMPs, TIMP-1 was chosen as it was previously analyzed in several clinical studies. Recently, we confirmed an inverse correlation for MMP1/TIMP1 ration both at implants and natural teeth [50]. MMP-1 is known to be higher in GCF in periodontal disease, after therapy levels decrease with a lower ratio to TIMP-1 [51]. In this study, levels of MMP-1 decreased only in patients highly responsive to periodontal therapy, and TIMP-1 was not detectable by the used method.

The used GLM model underlines an overwhelming role of *P. gingivalis*. Significant differences between the low and high responders were found 3 months after SRP; the detection of *P. gingivalis* decreased significantly only in the HR group. Real-time PCR of *F. alocis* was added as it was among the species differentiating between good and low responders by using a microarray with 300 species [52]. Our results of a reduction only in the HR group may confirm this. Except for *F. alocis*, all other species were analyzed by using a commercially available kit using strip technology. This kit has clearly defined cutoffs for the analyzed species, and validating qualitative data seems to be sufficient. Additional information seems to be limited when using categorial data. The sum of major bacteria being associated with periodontal disease decreased in HR group at 3- and 6-month appointments. For second-line bacteria, no clear significance was seen. In our chronic periodontitis patients, initial detection of *A. actinomycetemcomitans* was not linked with low response. This species was more present in the HR group at baseline, while in the LR group, an increase at later appointments in comparison with baseline was observed. In Chinese chronic periodontitis patients, clinical response to periodontal therapy did not depend on initial detection of *A. actinomycetemcomitans* [53]. Although recently, no benefit of microbial analysis was seen in treatment evaluation [54], the reduction of major bacteria being associated with periodontal disease in good responders is in accordance with most other studies [16, 52, 55, 56]. Thus, our data may support the importance of the microbiological analysis of key microorganisms as potential predictors for treatment outcome.

In summary, if microbiological diagnostics identifies *P. gingivalis* before SRP, an additional subgingival biofilm sample taken 3 months after SRP and analyzed for that species may have a predictive value in response to periodontal therapy. The combination with MMP-8 in oral lavage or preferably calprotectin in GCF might give additional information; however, for determination of calprotectin, an easy-to-perform standardized test is needed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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