A study into systemic and oral levels of proinflammatory biomarkers associated with endpoints after active non-surgical periodontal therapy

Werner N, Frasheri I, Heck K, Scalia C, Pitchika V, Summer B, Ern C, Heym R, Schwendicke F, Bumm CB, Folwaczny M
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Compiled by Dr Sarah Chin

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Introduction

Periodontitis results from inflammation within the gingival tissues due to the presence of dysbiotic microbiota. The degree of immune response is influenced by genetic and epigenetic factors, as well as intrinsic and extrinsic exposures such as systemic disease. The majority of periodontitis associated biomarkers are cytokines and chemokines expressed by endothelia, epithelial cells, fibroblasts, macrophages and T-lymphocytes in response to the dysbiotic microbiota. Bacterial antigens (Pathogen associated molecular patterns - PAMPs), such as lipopolysaccharides, induce bioactive substance expression and can be detected in the serum (SE) and plasma (PL) of venous blood as well as gingival crevicular fluid (GCF).

Matrix metalloprotinease-8 (MMP-8) and prostaglandin E2 (PGE2) have been used as diagnostic marker molecules demonstrating inflammation levels and severity of periodontitis. Surfactant protein D (SP-D) is an innate immune scavenger receptor that binds to various PAMPs. It is present in plasma and has been shown to have antimicrobial and anti-inflammatory effects in cardiovascular and metabolic diseases. It also has been shown to reduce systemic inflammation in mouse models. Increased plasma concentration has been seen in periodontitis.

The current classification suggests the use of biomarkers to improve diagnostic accuracy. As there is insufficient evidence in the literature, this retrospective study was designed to assess the level of biomarkers - active MMP-8 (aMMP-8), PGE2 and SP-D in periodontitis patients and to analyse their association with the response to step I (preventive and health promotion) and II (cause-related therapy- elimination of subgingival biofilm and calculus) periodontal therapy and non- surgical re-instrumentation (NSRI).

Methods

Two hundred and nine patients were enrolled in step I and II periodontal therapy in the undergraduate course at the Department of Conservative Dentistry and Periodontology University Hospital, LMU Munich between February 2011 and March 2016.

Patients either received initial treatment for initially diagnosed periodontal disease or re-treatment of recurrent disease. The inclusion criteria were: age of eighteen years and above; diagnosis of periodontitis based on the current classification; periodontal chart including probing pocket depth, bleeding on probing at six sites per tooth at baseline, at re-evaluation and NSRI; laboratory analysis of SE-aMMP-8, GCF-aMMP-8, GCF-PGE2 or PL-SP-D at baseline; and willingness to provide informed consent. The exclusion criteria were pregnancy at baseline, prior periodontal treatment within the two years before study enrolment, current enrolment in supportive periodontal therapy, or administration of systemic antibiotics as an adjunct to step II therapy.

Periodontal treatment was performed by undergraduate students supervised by periodontists. Step I therapy involved patient education in regard to aetiology, pathogenesis, risk factors and treatment plan, oral hygiene instruction and professional plaque removal. Step II therapy involved professional plaque removal and subgingival debridement was performed under local anaesthesia at all teeth with PPD>3mm using sonic devices and Gracey curettes. NSRI was performed at persisting pockets with PPD of 4mm with BOP and ≥5mm at re-evaluation similar to that described for step II therapy.

Clinical and radiographic examinations (where indicated) were conducted prior to steps I and II therapy (baseline, T0), 6 months after therapy (T1) and after NSRI (T2). PPD and BOP at six sites/

tooth were measured. Mobility was measured using Miller's index and furcation was measured by Hamp classification with a Naber's probe. Staging, grading and extent of disease were measured for each patient as by Tonetti (2018). The proportion of periodontal pockets was defined as the percentage of sites with PPD ≥4mm at baseline and PPD =4mm with BOP or PPD ≥5mm at T1. The 'treat to target' endpoint (T2T) was defined as ≤4 sites with PPD ≥5mm. Smoking status was classified as smoking or non-smoking.

The teeth were air-dried and isolated with cotton rolls. GCF strips were inserted into the bottom of the pocket and held in place for 30s and then collected in cryotubes. GCF was collected from the deepest periodontal pocket in each quadrant using sterile GCF/PISF (peri-implant sulcular fluid) collection strips and pooled per patient for analysis. Blood samples were collected in four tubes: two EDTA tubes for plasma and two serum tubes. aMMP-8 levels were quantified in serum and GCF using the dentoELISA aMMP-8 ELISA kit while PGE2 levels were assessed using PGE2 high sensitivity EIA kits. Plasma samples were analysed using the Human Surfactant Protein D ELISA.

Sample size calculations were performed and a minimum of 104 subjects were required to be enrolled for a power of 0.9.

Results

Two hundred and nine patients met the inclusion criteria from 759 patients who received steps I and II therapy between February 2011 and March 2016. The mean patient age was 59 ±11 years. At baseline, patients presented with 23 ±6 teeth, 23.9% of subjects were smokers and 8.6% had diabetes. Stage II periodontitis was diagnosed in 5.7% of patients, 76.1% had stage III and 18.2% had stage IV. A majority of patients had generalised disease (82%) and 4.8% were classified as grade A, 59.3% as grade B and 35.9% as grade C. The mean PPD was 2.78 ±0.56mm.

Patients presented for T1 6.33 ±3.79 months after step II therapy and for T2 5.93 ±4.31 months after NSRI. At T1, all patients had a lower proportion of sites with periodontal pockets compared with baseline (21.5 ±15.4% at T0 vs 9.9 ±9.6% at T1); 41.6% reached T2T at T1. Of those who failed to reach T2T, 26.5% were smokers and 9.6% had diabetes. After NSRI, patients who reached T2T increased to 47.4% and there was a further reduction of proportion of sites with periodontal pockets (8.2 ±7.7%) at T2.

Periodontal treatment resulted in a significant reduction of GCF aMMP-8 compared with baseline, T1 and T2. SE-aMMP-8 and GCF-PGE2 were unchanged. PL-SP-D increased significantly after treatment. Levels of biomarkers did not differ between patients achieving T2T or not at T1. See Table 2 for further details.

A potential association between therapeutic outcomes after steps I and II therapy and baseline PL-SP-D (OR 0.559) was observed. No potential association was found for therapy outcome after NSRI. Multivariate analysis indicated a 57% reduction in the risk of not reaching T2T at T1 when adjusting for sex, diabetes, mean baseline PPD and current smoking status. Neither combined blood biomarkers or the GCF biomarkers at T1 or T2 showed a significant association with therapy outcomes after steps I and II therapy and NSRI.

Discussion

The results from this study indicate a potential association between higher baseline PL-SP-D concentration and a better treatment outcome after steps I and II therapy. Oral biomarkers and markers of the innate immune response are associated with periodontal disease and treatment outcomes.

PAMPs have been shown to be elevated in the presence of distinct bacterial antigens. Several biomarkers are associated with higher severity of disease, but only some of these markers can reliably predict disease progression or treatment outcome. Biomarkers can be found in venous blood, GCF or saliva. There can be issues with collection (GCF), lower biomarker level (saliva) and influence of systemic factors. Blood biomarkers reflect the host's systemic response to treatment more accurately than salivary biomarkers.

Using the endpoint of T2T allows for comparison of treatment response at the patient level and reflects control of the chronic inflammatory process. Most of the patients (58.4%) were unable to reach T2T. Smokers had a poorer response to steps I, II therapy and NSRI which is similar to other studies where 27% of patients had successful treatment without systemic antimicrobial treatment. Other studies have suggested entirely stable periodontitis may be difficult to reach in patients with stages III and IV after non-surgical therapy which is similar to the results of this study showing only 1.0% of patients had complete pocket closure at re-evaluation.

TABLE 2 | Biomarker concentrations during therapy.

Factor	*n*	Baseline (T0)	After steps I and II therapy (T1)	After non-surgical re-instrumentation (T2)	*p*-value
Serum aMMP-8 (ng/mL)	163	86 [47–155]	79 [43–132]	79 [50–133]	0.620
GCF aMMP-8 (ng/mL)	176	175 [76–355]	138 [57–261]	129 [57–225]	**0.013**[a,b]
GCF PGE2 (pg/mL)	174	98 [52–184]	74 [52–111]	81 [53–157]	0.060
Plasma SP-D (ng/mL)	171	134 [87–199]	142 [100–219]	162 [113–219]	**<0.001**[a]

Note: Data are presented as median [q1–q3]. Comparisons are analysed using the Friedman test. Bold indicates statistically significant values ($p < 0.05$).
Abbreviations: aMMP-8, active matrix metalloproteinase 8; GCF, gingival crevicular fluid; PGE2, prostaglandin E2; SP-D, surfactant protein D; T0, prior to steps I and II therapy; T1, after steps I and II therapy; T2, after nonsurgical re-instrumentation.
[a]T0 versus T1, $p < 0.05$.
[b]T0 versus T2, $p < 0.05$.

Only higher PL-SP-D levels at T0 associated with a considerably reduced risk of treatment failure after steps I and II therapy. SP-D has been shown to reduce neutrophil-induced oxidative stress in other inflammatory diseases and suppress the formation of neutrophil extracellular traps which have been associated with periodontitis and may also promote neutrophil mediated clearance of bacteria. SP-D binds to gram negative bacteria through the recognition of lipopolysaccharides.

PGE-2 and aMMP-8 concentrations at baseline did not differ significantly between patients with and without successful therapy according to T2T criteria after steps I and II therapy and NSRI. This study showed other marker molecules may be more closely related to individual therapy response than aMMP-8. This study showed no association between salivary PGE2 and success of periodontal treatment. Other studies have shown that periodontitis related osteoclast activation is not dependent on PGE2 but on IL-1ß and IL-6 and RANKL. Combination of various marker molecules not included in this study may help with diagnostic accuracy in the future.

Limitations of this study include being a single-centre study and stage I and II therapy and NSRI being completed by undergraduate students, which may be a limitation regarding the comparability of the data, reflecting the range of treatment achieved in normal clinical setting. Biomarker levels in the literature are highly heterogenous. Many studies have used a dichotomous point of care test instead of metric concentrations for aMMP-8.

Conclusion

This study indicates that besides PPD at baseline and smoking, PL-SP-D levels may be associated with therapy outcomes after steps I and II of periodontal therapy. No association was found for PL-SP-D and NSRI. From this study, higher baseline PL-SP-D levels were associated with a more favourable treatment outcome after steps I and II therapy.

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Questions:

1. Which of the inflammatory biomarkers showed a potential association for a therapeutic outcome after steps I and II therapy from baseline:
   a. Serum aMMP-8
   b. GCF aMMP-8
   c. GCF PGE2
   d. Plasma SP-D

2. Treat to target endpoint (T2T) was defined as:
   a. ≤4 sites with PPD ≥5mm
   b. ≤4 sites with PPD ≤5mm
   c. ≤5 sites with PPD ≤4mm
   d. ≥4 sites with PPD ≥5mm

3. Periodontal treatment resulted in a significant reduction of GCF PGE2 compared with baseline, T1 and T2. TRUE or FALSE.

4. At T1, what proportion of patients reached T2T:
   a. 9.9%
   b. 21.5%
   c. 41.6%
   d. 47.4%

5. One of the limitations of this study include:
   a. Periodontists performed the treatment only at NSRI
   b. Undergraduate students performed the periodontal treatment
   c. PGE2 and aMMP8 were used as a marker of inflammation
   d. Patient numbers were underpowered

6. The rate of complete pocket closure in stage III and IV periodontitis was:
   a. 58.4%
   b. 57%
   c. 27.0%
   d. 1.0%

7. Neither combined blood biomarkers or the GCF biomarkers at T1 or T2 showed a significant association with therapy outcomes after steps I and II therapy and NSRI.
   TRUE or FALSE.

8. Compared with baseline, periodontal treatment resulted in a significant reduction of:
   a. SE-aMMP-8
   b. GCF- PGE2
   c. GCF aMMP-8
   d. SE- PGE2

9. Periodontitis related osteoclast activation has been shown in other studies to be dependent on:
   a. PGE2
   b. RANKL
   c. aMMP-8
   d. IL-1

10. PAMPs are produced by:
    a. Bacteria
    b. Epithelial cells
    c. T- lymphocytes
    d. Macrophages

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