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Original Article

MMP-8 and TIMP-1 are associated to periodontal inflammation in patients with rheumatoid arthritis under methotrexate immunosuppression – First results of a cross-sectional study

G. Schmalz [a,e], I. Davarpanah [a,e], J. Jäger [b], R.F. Mausberg [b], B. Krohn-Grimberghe [c], J. Schmidt [a], R. Haak [a], U. Sack [d], Dirk Ziebolz [a,*]

[a] *Dept. of Cariology, Endodontology and Periodontology, University of Leipzig, Germany*
[b] *Dept. of Preventive Dentistry, Periodontology and Cariology, University Medical Center Goettingen, Germany*
[c] *Clinic of Rheumatology, Bad Wildungen, Germany*
[d] *Institute of Clinical Immunology, Medical Faculty, University of Leipzig, Germany*

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Abstract *Background:* Aim of this cross-sectional study was the investigation of associations between different rheumatoid arthritis (RA)-related blood parameters and periodontal condition as well as selected periodontal pathogenic bacteria in RA patients under methotrexate (MTX) immunosuppression.
Methods: Periodontal probing depth (PPD), bleeding on probing (BOP) and clinical attachment loss (CAL) were assessed. Periodontal condition was classified into: no/mild and moderate or severe periodontitis (P). Prevalence of selected periodontal pathogenic bacteria and concentration of matrix metalloproteinase 8 (MMP-8) was assessed from the gingival crevicular fluid (GCF) using PCR and ELISA, respectively. Blood samples were analyzed for the concentration of selected rheumatoid parameters. Statistical analysis: t-test, Mann–Whitney-U-Test, exact Fisher tests or chi square test ($p < 0.05$).
Results: Fifty-six patients (mean age 55.07 years, 34 P, 22 no P) were included. While prevalence of periodontal pathogenic bacteria was higher in P patients, no substantial association

* Corresponding author. University Medical Center Leipzig, Dept. of Cariology, Endodontology and Periodontology, Liebigstr. 10-14, D 04103 Leipzig, Germany. Fax: +49 341 97 21219.
E-mail address: dirk.ziebolz@medizin.uni-leipzig.de (D. Ziebolz).
[e] These authors contributed equally to this work.

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of bacteria with blood parameters was found. In periodontal diseased participants, MMP-8 concentration in GCF (6.22 ± 7.01 vs. 15.99 ± 13.49; $p < 0.01$) and blood (2.60 ± 3.57 vs. 5.52 ± 5.92; $p < 0.01$) was increased, while no correlation between GCF and blood was found (Spearman's rho: 0.175; $p = 0.23$). Furthermore, higher blood concentrations of MMP-8 and tissue inhibitor of MMP (TIMP-1) were detected in patients with increased periodontal inflammation (BOP positive, $p < 0.01$).

Conclusion: Periodontal inflammation appears associated to MMP-8 and TIMP-1 in blood. Thereby, clinical interaction between periodontal conditions, periodontal pathogenic bacteria and RA-related cytokines remain unclear.

Introduction

Periodontitis (P) and rheumatoid arthritis (RA) are chronic inflammatory diseases, which are extensively discussed to stand in an interrelationship.[1] A dysregulation of the immune system, leading to the destruction of soft and hard tissues appears to be causative in these two diseases.[2] However, the causality of the interrelation between P and RA has not been completely clarified, yet.[3–7]

Although P is primary caused by bacteria, the disease is multifactorial and therefore complex.[8] Additionally, the pathophysiology of RA has a very high complexity, which up until now has not been fully understood.[9,10] On the one hand, periodontal pathogenic bacteria might be involved in the interaction between P and RA.[11–13] Thereby, the role of *Porphyromonas gingivalis* is the most investigated issue in this context.[13–15] On the other hand, markers for the host response e.g. matrix-metalloproteinase 8 (MMP-8) and tissue inhibitor of MMP (TIMP-1) might be involved in the complex interrelationship.[16,17] Therefore, the clinical investigation of periodontal pathogenic bacteria and these inflammatory markers might help to understand the relation between P and RA. Furthermore, it has been shown that similar cytokines are involved in P and RA.[18] Consequently, different RA related blood parameters might be influenced by periodontal inflammation and vice versa.

One important aspect in this context is the heterogeneity within RA patients, especially regarding their medication with immunosuppressive drugs, including different disease modifying anti-rheumatic drugs (DMARD) as monotherapy or combination.[10] Previous studies by this working group, which investigated periodontal situation, periodontal pathogenic bacteria and inflammatory markers in RA patients, also pointed this heterogeneity.[19–21] Especially periodontal inflammation, but also inflammatory markers might be influenced by different immunosuppressive medications. Furthermore, it was shown that periodontal condition might be influenced by antirheumatic medication.[22] Consequently, an investigation of these parameters in RA patients might be more meaningful if the patients were separated by their medication.

From the large number of DMARD, methotrexate (MTX) is one of the preferred drugs because of its excellent benefit to toxicity profile.[23,24] MTX works via multiple mechanisms including adenosine mediated anti-inflammatory effects, increased apoptosis of T-cells and the reduction of cell proliferation.[25,26]

To the best of the authors' knowledge, there is no study available, which investigated the periodontal situation in RA patients, which were exclusively treated with MTX. Consequently, the aim of the current study was the investigation of associations between different RA-related blood parameters and periodontal condition as well as selected periodontal pathogenic bacteria in RA patients under MTX immunosuppression. It was hypothesized that the presence of a periodontal disease would be associated to RA-related blood parameters in RA patients under MTX medication.

Material und methods

Study design

The current investigation was carried out as a clinical monocentric cross-sectional study. It was reviewed and approved by the ethics committee of the medical faculty of the Georg-August-University, Goettingen, Germany (application no. 9/4/11). The patients were informed verbally and in writing about the study, and they gave written informed consent.

Patients

The patients were selected from a pool of patients who were undergoing treatment and routine follow-up controls in the rheumatology clinic Bad Wildungen, Hessen/Germany. The following inclusion criteria were determined: age between 18 and 70 years and a defined medical diagnosis of RA (criteria of the American College of Rheumatology = ACR) established by a rheumatologist (BK-G).[27] In addition, a maximum period since initial RA diagnosis of 5 years was defined as an inclusion criterion. Furthermore, only patients with immunosuppressive medication with methotrexate (MTX) were included.

The exclusion criteria were as follows: additional general disorders and diseases such as diabetes mellitus, infectious diseases (hepatitis, HIV), seizure disorders or neuropathy, addictive disorders, renal disorders and organ transplants, as well as pregnancy.

Furthermore, the following data were recorded from the medical records of the participants: age, gender, general and RA-specific medication, smoking habits (smoker or not; definition of nonsmoker: never smoking or nonsmoker for a period that was longer than the past 5 years), disease duration and the current disease activity score (DAS28-ESR). Additionally, the current blood parameters C-reactive protein (CRP), antibody against cyclic citrullinated peptide (CCP) and rheumatoid factor (RF) were recorded.

Periodontal examination

One experienced and calibrated dentist performed periodontal examination. Gingival inflammation was evaluated using the papilla bleeding index (PBI) with a scoring ranged from 0 (no bleeding/inflammation-free gingiva) to 4 (profuse bleeding/severe inflammation), using a periodontal probe (PCP 15; Hu-Friedy, Chicago, IL, USA). The following periodontal parameters were assessed on six measurement points per tooth using a millimeter-scaled periodontal probe (PCP 15; Hu-Friedy, Chicago, IL, USA): periodontal probing depth (PPD), bleeding on probing (BOP) and clinical attachment loss (CAL). According to the definition of AAP/CDC, periodontal condition was classified based on the PPD and/or CAL in three categories: no or mild, moderate and severe periodontitis.[28] Based on this classification, patients with no or mild P was assigned to the non-P group, while patients with moderate or severe P were assigned to P group.

Analysis of MMP-8 and selected periodontal pathogenic bacteria

At first, supragingival plaque was removed. Then, gingival crevicular fluid samples for MMP-8 analysis were taken from $\geq$2 of the deepest periodontal pockets (maxilla and mandible) using sterile paper points. The paper points were placed in the sulcus for 30 s and pooled for examination. The aMMP-8 level of each patient was determined using an enzyme-linked immunosorbent assay (ELISA) with reliable, validated monoclonal antibodies from a commercial test system (quantitative aMMP-8-laboratory test, Dentognostics, Jena, Germany) in the clinical laboratory of the Department of Preventive Dentistry, Periodontology and Cariology, University Medical Center Goettingen.

Following sample collection for MMP-8 detection, subgingival biofilm samples from the sulcus fluid of the same pockets were taken for microbiological diagnostic. These paper points were placed in the sulcus for 20 s and pooled for examination. Investigation of DNA of selected periodontal pathogens was carried out using Polymerase-Chain-Reaction (PCR) with a commercially available detection kit (micro-IDent®plus, Hain Lifescience GmbH, Nehren, Germany). The following 11 periodontal pathogenic bacteria were examined: *Aggregatibacter actinomycetemcomitans (Aa), P. gingivalis (Pg), Tannerella forsythia (Tf), Treponema denticola (Td), Prevotella intermedia (Pi), Parvimonas micra (Pm), Fusobacterium nucleatum (Fn), Campylobacter rectus (Cr), Eubacterium nodatum (En), Eikenella corrodens (Ec), Capnocytophaga species (Cs)*.

Analysis of specific inflammatory blood parameters

Blood samples were obtained during routine check-ups. Venous blood samples were collected under sterile conditions. Concentrations of transforming growth factor β1 (TGF-β1; R&D Systems, detection limit: 4.61 pg/ml), interferon-gamma (IFN-g; R&D Systems, detection limit 8 pg/ml), interleukin-6 (IL-6; R&D Systems, detection limit: 0.039 pg/ml), IL-23 (R&D Systems, detection limit: 6.8 pg/ml), TIMP-1 (R&D Systems, detection limit: 0.08 ng/ml), and MMP-8 (R&D Systems, detection limit: 0.02 ng/ml) were analyzed according to manufacturer's instructions.

Statistical analysis

The statistical analyses were carried out by means of SPSS for Windows, version 22.0 (SPSS Inc. US). The continuous variables were presented as means and medians, while standard deviations were chosen as measure of dispersion. Normal distribution was tested using Kolmogorov–Smirnov-Test ($p < 0.05$).

For the comparison of two independent, normally distributed samples, the t-test was applied, for non-normally distributed samples the Mann–Whitney-U-Test was applied as a non-parametric procedure. The categorized data, on the other hand, was evaluated by means of the exact Fisher tests or chi square test, whereby all necessary requirements for these tests were fulfilled. The significance level was set at $\alpha = 5\%$.

Results

Patients

Fifty-six patients (mean age 55.07 $\pm$ 11.75 years, female: 27) with RA under MTX immunosuppression were included. The period since RA-diagnosis was 2.34 $\pm$ 2.67 years for all patients, and the current DAS28-ESR was 3.52 $\pm$ 1.42 (Table 1). After separation of non-P and P group within RA patients under MTX medication, patients with P had a higher mean age compared to non-P group (49.59 $\pm$ 11.76 vs. 58.62 $\pm$ 10.45; <0.01). Further characteristics of all patients are given in Table 1.

Periodontal findings

Periodontal findings including PBI, PPD, BOP, CAL and P severity are shown in Table 2. Sixty-one percent of the patients (n = 34) were found to have a moderate to severe P, according to the classification of the AAP/CDC.[28]

Microbiological findings

The prevalence of selected periodontal pathogenic bacteria is shown in Fig. 1A. The proportion of positive bacterial findings of all selected periodontal pathogenic bacteria was higher in P patients compared to patients without P (Fig. 1B). Thereby, the results for *Pg* ($p = 0.02$), *Tf* (<0.01), *Td* ($p < 0.01$) and *Cs* ($p = 0.04$) were statistically significant, while for *Aa* ($p = 1.00$), *Pi* ($p = 0.17$), *Pm* ($p = 0.27$),

Table 1 Patient's characteristics of all patient groups as well as of patients with and without periodontitis.

Characteristic		All (n = 56)	Non-periodontitis (n = 22)	Periodontitis (n = 34)	p-Value non-PD vs. PD
Gender [n]	Female	27	12	15	0.59
	Male	29	10	19	
Age in years [mv ± sd]		55.07 ± 11.75	49.59 ± 11.76	58.62 ± 10.45	**<0.01**
Smoking habits [n]	Non smoker	41	19	26	0.50
	Smoker	15	3	8	
Period since initial diagnosis in years (mv ± sd)		2.34 ± 2.67	1.87 ± 2.57	2.65 ± 2.74	0.16
Specific rheumatology parameters	Current DAS28-ESR [mv ± sd]	3.52 ± 1.42	3.60 ± 1.39	3.47 ± 1.45	0.75
	RF positive [n]	18	7	11	0.77
	Current CCP positive [n]	17	5	12	0.75

mv: mean value, sd: standard deviation, CRP: C-reaktives Protein, WBC: leukocytes, PLT: thrombocytes.
Significant results ($p < 0.05$) are highlighted in bold.

Table 2 Periodontal parameters in the patient groups.

Parameter		Total (n = 56)	Non-periodontitis (n = 22)	Periodontitis (n = 34)
PPD in mm (mv ± sd)		3.15 ± 1.11	2.60 ± 0.65	3.54 ± 1.20
BOP in % (mv ± sd)		27.43 ± 29.45	6.44 ± 13.61	41.01 ± 29.04
CAL in mm (mv ± sd)		3.35 ± 1.29	2.74 ± 0.76	3.79 ± 1.41
PBI (mv ± sd)		0.10 ± 0.18	0.10 ± 0.13	0.10 ± 0.23
Periodontal condition (n) [%]	No/mild periodontitis	22 (39)	22 (100)	0
	Moderate periodontitis	27 (48)	0	27 (79)
	Severe periodontitis	7 (13)	0	7 (21)

mv: mean value, sd: standard deviation, PPD: periodontal probing depth, BOP: bleeding on probing, CAL: clinical attachment loss, PBI: papilla bleeding index.

Fn ($p = 0.15$), *Cr* ($p = 0.17$), *En* ($p = 0.70$) and *Ec* ($p = 0.60$) no statistical significance was detected.

Association of periodontal disease with blood parameters

Both, the GCF- (6.22 ± 7.01 vs. 15.99 ± 13.49; $p < 0.01$) and the blood-concentration of MMP-8 (2.60 ± 3.57 vs. 5.52 ± 5.92; $p < 0.01$) were significantly higher in P group (Table 3). Furthermore, the presence of a moderate to severe P showed no significant association to further blood parameters (Table 3).

Association of different factors with blood parameters

Periodontal pathogenic bacteria

The presence of *Aa* was statistically significant associated to TGF-β1 and IL-6 ($p = 0.04$). Furthermore, *Pm* showed a significant association to IL-6 ($p < 0.01$), while for *Fn* a significant association to TIMP-1 ($p = 0.04$) and IL-23 ($p = 0.01$) was detected (Table 4). Significant results for further selected periodontal pathogenic bacteria on the investigated blood parameters were not found ($p > 0.05$; Table 4).

Periodontal inflammation (BOP positive)

Higher BOP values showed significantly increased TIMP-1 ($p < 0.01$; Fig. 2A) and MMP-8 ($p < 0.01$; Fig. 2B) concentration in blood. No association of BOP values were detected with IFN-γ ($p = 1.00$), TGF-β1 ($p = 0.94$), IL-23 ($p = 0.10$) and IL-6 ($p = 0.80$).

MMP-8 concentration in GCF

No correlation was found between GCF- and blood-concentration of MMP-8 (Spearman's rho: 0.175; $p = 0.23$). Furthermore, no association of MMP-8 concentration in GCF was found to TIMP-1 ($p = 0.15$), IFN-γ ($p = 1.00$), TGF-β1 ($p = 0.73$), IL-23 ($p = 0.17$) and IL-6 ($p = 0.55$).

Discussion

Summary of the main results

The current study's aim was to investigate associations between different blood parameters and periodontal condition as well as selected periodontal pathogenic bacteria in RA patients under MTX immunosuppression. While prevalence of selected periodontal pathogenic bacteria was higher in P patients, no substantial association between the bacteria and blood parameters was found. In periodontal

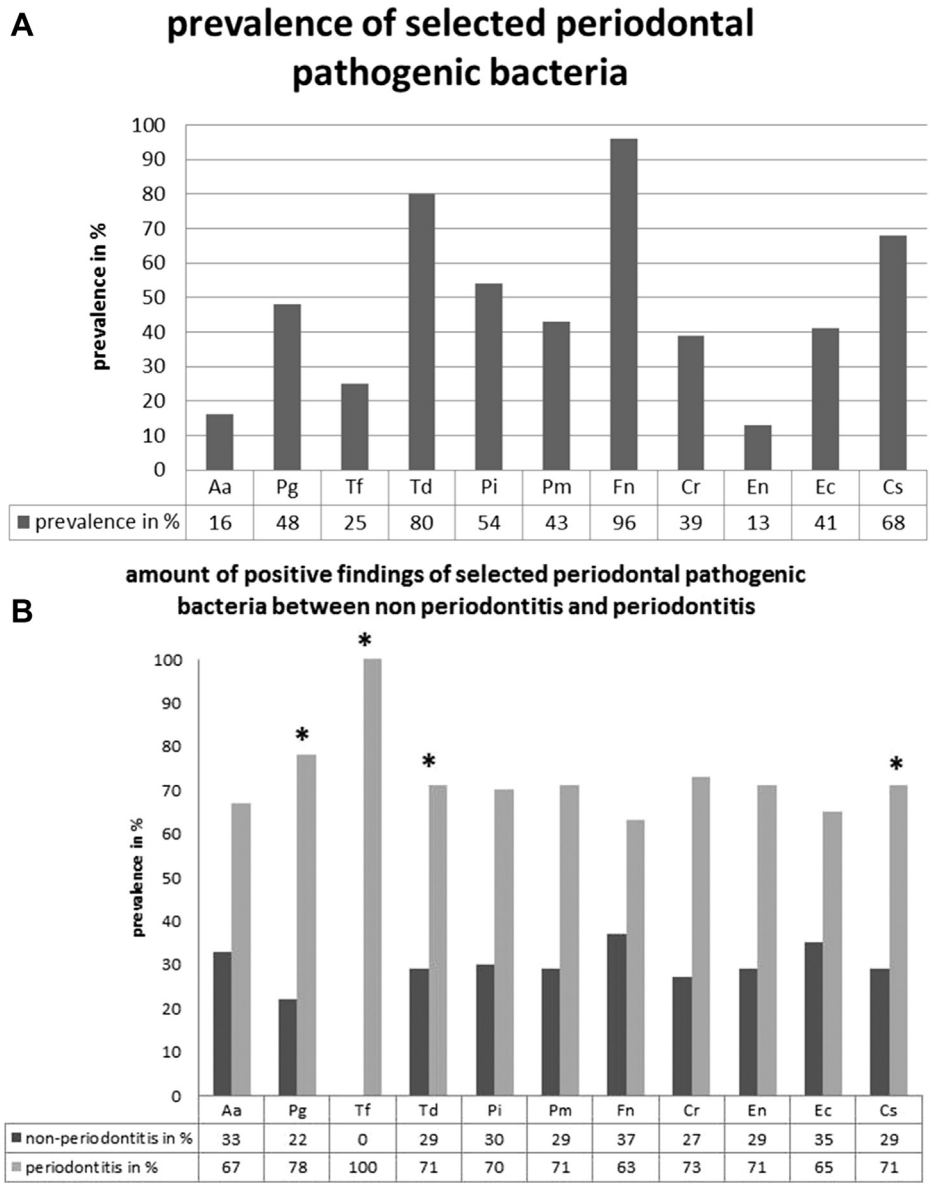

Figure 1. A: Prevalence of selected periodontal pathogenic bacteria in all patients. B: Comparison of the proportion of positive findings of selected periodontal pathogenic bacteria between periodontitis and non-periodontitis patients. *Significant difference ($p < 0.05$).

diseased participants, MMP-8 concentration in blood and GCF was increased. However, a correlation of the MMP-8 concentration in blood and GCF could not be found. Furthermore, higher blood concentrations of MMP-8 and TIMP-1 were detected in patients with increased periodontal inflammation (BOP positive).

Interpretation of the results and comparison with the literature

To the best of the authors' knowledge, this is the first study, which investigated the association of periodontal parameters with selected periodontal pathogenic bacteria and blood parameters in rheumatoid arthritis patients under MTX medication. RA is a very heterogeneous and complex disease. Many immune cells (eg, T-helper-1 and T-helper-17 cells, B cells, plasmablasts, and plasma cells) and cytokines (e.g. interleukins) are involved in its pathophysiology.[29] The current study selected just few of the variety of cytokines. Therefore, IL-6, IL-23, TGF-β1 and IFN-γ as well as MMP-8 and TIMP-1 were chosen. These cytokines were all reported to be related to different disease processes in RA like angiogenesis, bone metabolism, joint damage as well as T cell related induction, chronicity and relapse of the disease.[30—34] These cytokines are also involved in the process of periodontal inflammation and damage.[22,35—37] Accordingly, it appeared to be a meaningful approach to investigate these cytokines in blood between non-P and P patients with RA. Of course, these cytokines are only a small unspecific extract of the complex and comprehensive range of potentially relevant cytokines. This must be considered in the interpretation of the results.

Table 3 Comparison of different blood parameters and MMP-8 concentration in gingival crevicular fluid between non-periodontitis and periodontitis patients. Values are given as mean ± standard deviation.

Parameter	No periodontitis (n = 22)	Periodontitis (n = 34)	p-Value
GCF MMP-8	6.22 ± 7.01	15.99 ± 13.49	**<0.01**
Serum MMP-8	2.60 ± 3.57	5.52 ± 5.92	**<0.01**
TIMP-1	134.81 ± 65.38	157.90 ± 94.04	0.33
IFN-γ	4.00 ± 0	4.00 ± 0	1.00
TGF-β1	3784.43 ± 1800.01	3779.05 ± 1669.50	0.71
Il-23	4.76 ± 5.65	9.73 ± 15.84	0.25
Il-6	2.28 ± 2.17	2.16 ± 1.64	0.76
TNF-α	2.62 ± 4.17	1.89 ± 1.67	0.66
CRP	0.71 ± 0.85	0.77 ± 1.33	0.39
WBC	6.88 ± 1.71	7.04 ± 1.85	0.75
PLT	263.14 ± 73.14	262.79 ± 59.92	0.99
Lymphocytes	30.09 ± 10.04	28,65 ± 9.00	0.62
Seg. granulocytes	59.95 ± 9.56	61.39 ± 11.59	0.67

GCF: gingival crevicular fluid, MMP: matrix-metalloproteinase, TIMP: tissue inhibitor of MMP, TGF-β1: transforming growth factor β1, IFN-γ: interferon-gamma, Il-6: interleukin-6, IL-23: interleukin 23, CRP: c-reactive protein, WBC: leukocytes, PLT: thrombocytes; significance level $p < 0.05$.
Significant results ($p < 0.05$) are highlighted in bold.

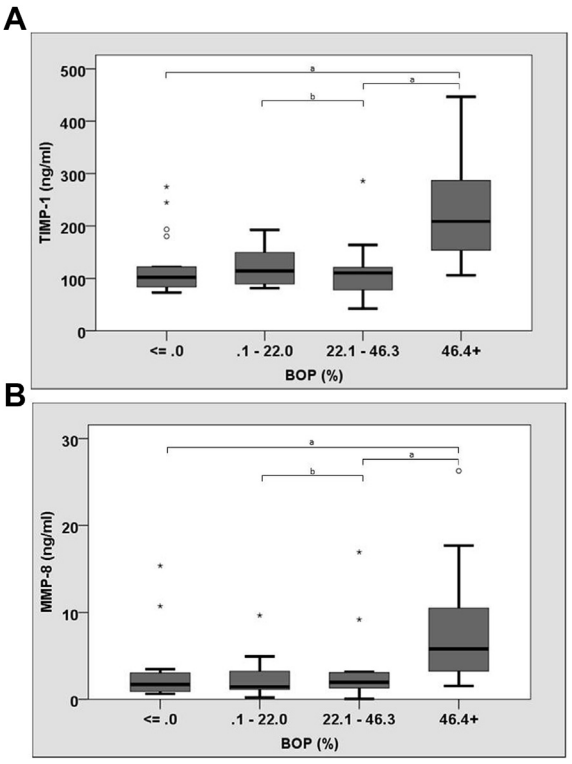

Figure 2. A: The association of BOP with the blood concentration of TIMP-1. The BOP values are categorized by the mean values. The symbols * and º represent statistical outliers. a: $p < 0.01$, b: $p < 0.05$. B: The association of BOP with the blood concentration of MMP-8. The BOP values are categorized by the mean values. The symbols * and º represent statistical outliers. a: $p < 0.01$, b: $p < 0.05$.

Because of the heterogeneity of RA patients, which was also present in previous studies of this working group,[26,27] the authors decided to include only patients with MTX medication. MTX is one of the preferred drugs because of its excellent benefit to toxicity profile and the recommended drug of first choice,[23,24] what makes it reasonable to investigate patients with that immunosuppression. Of course, this patient selection limits the comparability of the current study's findings with any results of the international literature.

Patients with RA were shown to present a higher amount of periodontal inflammation.[38] It is known that periodontal inflammation might have an influence on peripheral blood parameters, although this is often in a limited extent.[39] The current study was not able to show differences between non-P and P patients with RA under MTX for all blood concentrations of cytokines except for MMP-8. For MMP-8, both the GFC and blood concentration was higher in RA patients with periodontitis. The increased GCF values are in line with a previous study by this working group, which showed higher MMP-8 values in periodontal diseased RA-patients.[21] In this context, a unique finding of the current study is the absence of correlation between GFC and blood-concentration of MMP-8. Therefore, an influence of the generally increased activity of MMP-8 in RA patients might not be the causal factor for increased oral MMP-8 activity

Table 4 P values showing the association of different periodontal pathogenic bacteria with rheumatoid blood parameters.

Bacteria	TIMP-1	MMP-8	IFN-γ	TGF-β1	IL-23	IL-6
Aa	0.15	0.40	1.00	**0.04**	0.36	**0.04**
Pg	0.17	0.33	1.00	0.47	0.59	0.62
Tf	0.56	0.16	1.00	0.47	0.85	0.79
Td	0.22	0.40	1.00	0.71	0.20	0.31
Pi	0.09	0.15	1.00	0.30	0.72	0.72
Pm	0.50	0.52	1.00	0.21	0.95	**<0.01**
Fn	**0.04**	0.13	1.00	0.16	**0.01**	0.68

MMP: matrix-metalloproteinase, TIMP: tissue inhibitor of MMP, TGF-β1: transforming growth factor β1, IFN-γ: interferon-gamma, Il-6: interleukin-6, IL-23: interleukin 23, Aa: Aggregatibacter actinomycetemcomitans, Pg: Porphyromonas gingivalis, Tf: Tanerella forsythia, Td: Treponema denticola, Pi: Prevotella intermedia, Pm: Parvimonas micra, Fn: Fusobacterium nucleatum; significance level $p < 0.05$.
Significant results ($p < 0.05$) are highlighted in bold.

and thus periodontal damage. However, increasing periodontal inflammation (BOP+) was found to be associated to higher MMP-8 concentration in the current study. These results appear contradictory, but they might also underline the lack of understanding of the complexity of the interrelationship between P and RA and the unknown impact of immunosuppression (in special case MTX) regarding this. The periodontal inflammation was also associated with TIMP-1 besides MMP-8. Accordingly, higher MMP-8 and higher TIMP-1 values were detected in patients with increased periodontal inflammation. Literature for comparison of this finding is not available, yet.

Regardless, an imbalance of MMPs and TIMP-1 in the synovial fluid and blood of RA patients has been shown.[40,41] Compared to this, high TIMP-1-values but low MMP-8 values were detected in the current study, which appears to be a balanced situation. This could be caused by the immunosuppressive action of MTX. This influence of the MTX medication might not be present in the periodontal tissue, resulting in a missing correlation between blood and GCF concentration of MMP-8. This might be caused by the complexity and multifactorial character of periodontal inflammation.[8] The overall clinical significance of changes in serum MMP-8 and TIMP-1 levels in periodontal inflammation in RA patients under MTX treatment could be high. Especially, since it is known that MMP-8 and TIMP-1 are involved in the pathogenesis of various diseases, including RA, inflammatory bowel diseases, pulmonary diseases or vascular diseases.[42–46] In the context of RA, TIMP-1 levels could be associated to periarticular bone loss.[43] It has been even shown that MMP-8 could be a predictor of mortality in RA patients.[47] Of course, the current study's findings are unable to support these results; however, changes in the MMP-8 and TIMP-1 balance might have large clinical consequences for the RA patients. Accordingly, further large sampled studies should be performed to derive the clinical influence of the current study's findings.

An additional question of the current study was the potential association of selected periodontal pathogenic bacteria with selected RA-related blood parameters. Periodontal pathogenic bacteria are known to be an important initiator of periodontal inflammation, although the role of singular bacteria is controversially discussed.[48,49] The bacteria included in the current study are common periodontal pathogenic bacteria with different pathogenic potential.[50] Moreover, some of these bacteria are discussed to be involved in the relationship between P and RA. Especially infections with *P. gingivalis* were reported to negatively influence RA,[13–15] but also a range of other bacteria might play a role in the interrelationship between P and RA.[51,52] Although few statistically significant results were found regarding this, the small size of the subgroups must be considered in interpretation of the results. Comparable literature is rare. The influence of *T. denticola* or *P. intermedia* on MMP-8 concentration, as described in recent literature could not be confirmed.[53,54] However, the significant association with *A. actinomycetemcomitans* and IL-6 level supports available findings from animal studies.[55,56] Further findings regarding periodontal bacteria and different blood parameters are not in line with any available literature. Accordingly, the current study was not able to find clear evidence for the influence of selected periodontal pathogenic bacteria on blood parameters, neither for P. gingivalis, nor for another selected periodontal pathogenic bacterium. This is in line with the previous studies of this working group, which investigated the influence of periodontal pathogenic bacteria on rheumatoid disease parameters like DAS28-ESR, CCP and RF.[20,21] Consequently, the role of individual periodontal bacteria and their clinical relevance in interrelationship between RA and P appear questionable.

Strengths and limitations

The current study provides new and unique results about the association of the periodontal condition and selected periodontal pathogenic bacteria with RA related blood parameters in RA patients under MTX immunosuppression. However, a critical analysis of the limitations is necessary. First, although the sample size is comparatively high, especially in the subgroups P and non-P are relatively small. Therefore, the power of the current study might be too low to allow strong conclusions, especially for bacterial findings. A power calculation was not performed, but it was tried to include as many patients as possible within the available patient group. Nevertheless, based on the aforementioned reasons, the results must be seen as preliminary. Furthermore, the heterogeneity between P and non-P group is a limitation. However, a matching was not possible within this cross-sectional study. Similarly, the lack of a control group limits this investigation. However, an adequate control would be a group of patients with untreated RA, which is difficult to recruit. Beside of this, the focus was not to investigate the influence of the MTX medication, but on the comparison between patients with and without periodontitis within a group of RA patients, which should be as homogeneous as possible. For this, only patients with the same immunosuppression were chosen to avoid any differences caused by heterogeneous medication. Therefore, neither a healthy nor an untreated control group would bring much benefit in this question.

Furthermore, the selection of several blood parameters is a limitation of the current study. Many cytokines are involved in RA pathogenesis and it appears not possible to investigate all of them in one study, so a selection is needed. Similarly, the eleven selected periodontal pathogenic bacteria do not reflect the whole biofilm, which is involved in periodontal inflammation. The significant age difference between non-P and P group is another limitation of the study. Furthermore, the total amount of MMP-8 in GCF is more sensitive than expressing it as concentration.[57] However, the used test system in the current study only detected the concentration of MMP-8.

Additionally, there is very little comparable literature available. Therefore, an interpretation of the current studies results is difficult and largely speculative. Nevertheless, the results are new and a purposeful basis for future research.

Conclusion

While the GCF MMP-8 concentration did not correlate with the blood concentration, an association of periodontal inflammation with MMP-8 and TIMP-1 in the blood was

found. This might suggest a relation between periodontal inflammation and innate immune reaction in RA patients under MTX. The correlation between oral inflammation and changes in MMP-8 and TIMP-1 concentration in blood appears thereby unclear. Furthermore, the role of individual periodontal pathogenic bacteria and their clinical relevance in interrelationship between RA and P appear questionable.

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Conflict of interest

The authors declare no conflict of interest.

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