Periodontal pathogens participate in synovitis in patients with rheumatoid arthritis in clinical remission: a retrospective case-control study

Yuko Kimura¹, Shuzo Yoshida¹, Tohru Takeuchi¹, Motoshi Kimura², Ayaka Yoshikawa¹, Yuri Hiramatsu¹, Takaaki Ishida¹, Shigeki Makino¹, Yoshihiro Takasugi³ and Toshiaki Hanafusa¹

Abstract

Objective. The objective of this study was to investigate the role of periodontal pathogens in RA in remission.

Methods. Twenty-one patients with active RA and 70 patients in clinical remission, including 48 patients with synovitis [US power Doppler (USPD)(+) group] and 22 patients without synovitis [USPD(−) group] were clinically assessed by US. CRP, ESR, haemoglobin, MMP-3, RF and ACPA were measured. Antibody titres against four types of periodontal pathogen [Aggregatibacter actinomycetemcomitans, Eikenella corrodens (Ec), Porphyromonas gingivalis and Prevotella intermedia (Pi)] were analysed using ELISA.

Results. Musculoskeletal US examination showed that 68.6% of patients with RA in clinical remission exhibited synovitis. CRP, ESR, haemoglobin, MMP-3 and RF levels in both the USPD(+) and USPD(−) groups were clearly lower compared with the RA group in non-remission. The IgG serum antibody titre against Ec in the non-remission RA(+) group was significantly greater than that in the USPD(+) group, and the IgG antibody titre against Pi in the non-remission RA and USPD(+) groups was greater than in the USPD(−) group.

Conclusion. More than half of RA patients in remission showed persistent synovitis. This synovitis may be associated with periodontal disease-causing Pi. Thus, treating periodontal disease should also be considered in order to achieve more profound remission of RA.

Key words: rheumatoid arthritis, periodontitis, ultrasonography, power Doppler, clinical remission, periodontal pathogen.

Introduction

RA is a systemic inflammatory disease characterized by polyarthritis. Recently, it has become possible to suppress disease activity and to achieve clinical remission in many RA patients with early intervention using synthetic DMARDs such as MTX and biologic DMARDs [1-4].
However, both inflammation in the synovial tissues and progressive destruction of joints have been observed on musculoskeletal US in some RA patients in clinical remission [5–8]. It is unclear what factors contribute to the residual synovitis detected on musculoskeletal US.

Environmental factors such as smoking, periodontal pathogens and gut flora are known to be involved in the onset of RA [9–12]. In particular, there are numerous studies reporting a strong association between RA and periodontal diseases [13–15]. Joint tissue destruction in RA and periodontal tissue destruction in periodontal disease have been shown to exhibit similar pathologies and have therefore been suggested to have bidirectional causality [16]. *Porphyromonas gingivalis* (Pg) is the only oral bacterium that produces the enzyme peptidylarginine deiminase, and it has been suggested that it is involved in the production of ACPA [17, 18]. Patients with active RA have high serum IgG antibody titre against Pg [17, 18], thus suggesting involvement in active RA. Thus, periodontal pathogens may also be involved in the pathology of RA in clinical remission.

In this study, to investigate the role of periodontal pathogens in RA in remission, we examined the association of synovitis (detected by musculoskeletal US and serum IgG antibody titres) with periodontal pathogens in patients with RA in remission. Furthermore, we also investigated their association with established biomarkers for RA.

**Methods**

We investigated 91 patients with RA who received treatment on an outpatient basis at the Department of Internal Medicine (I), Osaka Medical College Hospital between September 2012 and August 2013. All patients met the ACR classification criteria for RA [19] and were evaluated by DAS28-CRP [20]. Patients who underwent RA treatment for ≥3 months and subsequently maintained remission (DAS28-CRP <2.3) for ≥6 months [21] were considered to have RA in clinical remission. Those who did not meet the DAS28-CRP-based remission criteria were considered to be RA in non-remission patients [21].

**Clinical assessment**

Information on gender, age, duration of disease and treatment drugs for RA (MTX, biologic drugs, non-MTX synthetic DMARDs, CSs, antibiotics and NSAIDs) were obtained from medical charts and RA patients. Two rheumatologists examined all patients and assessed tender and swollen joints. The DAS 28-CRP and DAS28-ESR [22, 23] were calculated for all RA patients. Evaluation with HAQ [24–26] was conducted in RA patients in clinical remission.

**Ultrasonographic assessment**

On the same day as physical examination, RA patients in clinical remission underwent US assessment by two sonographers. Twenty-eight joints (bilateral knees, shoulders, elbows, wrists, MCP joints 1–5 and proximal IP joints 1–5) were investigated by musculoskeletal US using both grey-scale (GS) and power Doppler (USPD) modes of an US diagnostic system (Viamo, Toshiba Medical Systems Corporation, Tochigi, Japan). Synovitis was assessed in the GS and USPD modes in accordance with the Outcome Measures in Rheumatology US Taskforce guidelines [27]. A semi-quantitative scoring scale (grade 0: absent; grade 1: mild; grade 2: moderate; grade 3: marked) [28] was used for each parameter. Total GS and USPD scores were calculated from the sum of scores for 28 joints [29].

**Laboratory determinations**

Using blood samples collected from all RA patients, CRP (reference value ≤0.25 mg/dl), ESR (0–14 mm/h), haemoglobin (Hb) (11.5–14.7 g/dl), MMP-3 (17.3–59.7 ng/ml), RF (≤15 IU/l) and ACPA (<4.5 U/ml) were measured.

The antibody titres against four types of periodontal pathogen [Aggregatibacter actinomycetemcomitans (Aa) and *Eikenella corrodens* (Ec), which are facultative anaerobes; Pg and *Prevotella intermedia* (P), which are obligate anaerobes] were measured using ELISA (Leisure Inc., Nagasaki, Japan), and are given as standardized values for all RA patients. In accordance with the protocols described by Sugi *et al.* [30], standardized values corrected for serum antibody titres of healthy subjects (age, 20–29 years) were calculated using the following equation.

Standardized value = IgG titre of patient–mean IgG titre of healthy subjects

S.D. determined by mean IgG titre of healthy subjects × 2

**Assessment of periodontal disease**

Number of teeth, mean probing pocket depths and clinical attachment level were measured in all RA patients by a highly trained dentist.

**Statistical analyses**

Categorical and quantitative variables were, respectively, described as number (%) and median (25th, 75th percentile). Data were analysed using Prism software 5.0 (Graph-Pad, San Diego, CA, USA). For group comparisons between two groups, the two-tailed Mann–Whitney test for skewed variables was used. For group comparisons between three or four groups, the Kruskal–Wallis test followed by Dunn’s multiple comparison test for skewed variables was performed. The Chi-squared test for independence was used to analyse contingency tables. A value of *P* < 0.05 was considered to be statistically significant.

All participants provided written informed consent prior to enrolling in the study, according to the Declaration of Helsinki (General Assembly October 2008), and this study was conducted with the approval of the Medical Ethics Committee of Osaka Medical College (Approval number:1103).
Results

The study included 21 patients with RA in non-remission (non-remission RA group) and 70 patients with RA in remission. Patients with RA in remission were divided into two groups depending on their total USPD score from musculoskeletal US examination; patients with total USPD score $\geq 1$ were considered positive for synovitis [USPD(+) group, 48 patients], and patients with a score of 0 were considered negative for synovitis [USPD(−) group, 22 patients]. One patient in the non-remission RA group was complicated with SS, but no patient had SS in the remission RA group.

Clinical assessment

Demographic data are shown in Table 1. There were no differences in age, gender or duration of RA between any of the groups. MTX was the most commonly used synthetic DMARD and was used for 62 RA patients (68%) in this study. There were no differences in MTX dose or percentage undergoing biologic DMARD therapy between the groups. Usage of synthetic DMARDs other than MTX was 76% for the non-remission RA group, which was greater than the 44% and 41% seen in the USPD(+) and USPD(−) groups, respectively ($P=0.0269$). Synthetic DMARDs other than MTX in this study included salazosulphapyridine (21 patients, 23%), tacrolimus (17 patients, 19%) and bucillamine (12 patients, 13%), and the usage frequencies of these medications did not differ between the groups ($P=0.1094$). The usage frequencies of prednisolone, antibiotics and NSAIDs were greater in the non-remission RA group when compared with the USPD(+) and USPD(−) groups ($P<0.0001$, $P<0.0001$, $P=0.0029$). Prednisolone is a risk factor for opportunistic infections, such as Pneumocystis jirovecii. To prevent infection with this pathogen, most patients received sulphadiazine–trimethoprim concomitantly with prednisolone.

Table 1 Demographic data from subjects with active RA or RA in clinical remission

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RA in non-remission ($n=21$)</th>
<th>RA in clinical remission</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), years</td>
<td>70 (64–75.5)</td>
<td>61 (48.5–69)</td>
<td>57 (54.5–69.5)</td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>17 (81)</td>
<td>35 (73)</td>
<td>19 (86)</td>
</tr>
<tr>
<td>Duration of RA, median (IQR), years</td>
<td>6 (2.75–14.5)</td>
<td>8 (4–15)</td>
<td>3 (1.75–8.5)</td>
</tr>
<tr>
<td>Medication use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTX, n (%)</td>
<td>11 (52)</td>
<td>38 (79)</td>
<td>13 (59)</td>
</tr>
<tr>
<td>Biologic DMARDs, n (%)</td>
<td>9 (43)</td>
<td>16 (33)</td>
<td>7 (32)</td>
</tr>
<tr>
<td>Non-biologic DMARDs, n (%)</td>
<td>16 (76)</td>
<td>21 (44)</td>
<td>9 (41)</td>
</tr>
<tr>
<td>Steroids, n (%)</td>
<td>14 (67)</td>
<td>7 (15)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Antibiotics, n (%)</td>
<td>11 (52)</td>
<td>6 (13)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>NSAIDs, n (%)</td>
<td>17 (81)</td>
<td>19 (40)</td>
<td>8 (36)</td>
</tr>
</tbody>
</table>

$P$-value by Kruskal–Wallis test or Chi-squared test. IQR: interquartile range; USPD (+): positive synovitis by power Doppler synovitis grade; USPD (−): negative synovitis by power Doppler synovitis grade.

Sulphadiazine–trimethoprim was given to 11 patients with remission RA (12%) and 9 patients with non-remission RA (43%). Evaluation of physical function by HAQ showed no differences between the USPD(+) and USPD(−) groups (Table 2). Both groups showed diminished scores for DAS28-CRP and DAS28-ESR when compared with the non-remission RA group ($P<0.001$).

Ultrasonographic assessment and laboratory determinations

Data on US assessment and serological indices are shown in Table 2. Synovitis assessment in GS mode showed that the total USPD score was lower in the USPD(−) group when compared with the USPD(+) group ($P<0.001$). CRP, ESR, Hb, MMP-3 and RF levels in both the USPD(+) and USPD(−) groups were clearly lower when compared with the non-remission RA group. CRP, ESR, Hb and MMP-3 levels in both the USPD(+) and USPD(−) groups were all within reference ranges. Median values of ACPA decreased in the order: non-remission RA group, USPD(+) group and USPD(−) group; however, all values were greater than reference values, and there were no differences between the three groups.

A comparison of IgG antibody titres against periodontal pathogens between groups is shown in Fig. 1. There were no differences in IgG antibody titre for Aa and Pg between any of the groups ($P=0.5553, 0.2488$). The IgG antibody titre against Ec was lower in the USPD(+) group than in the non-remission RA group; no differences were observed between the other groups. The IgG antibody titre against Pi in the USPD(−) group was clearly lower when compared with the non-remission RA and USPD(+) groups ($P=0.0388$, Mann–Whitney test).

Assessment of periodontal disease

There was no difference in number of teeth between the non-remission RA group, USPD (+) group and USPD (−) group [Median (25th centile, 75th centile): 22.5 (20.25, 25.75), 24.5 (15.0, 28.0) and 26.0 (21.5, 28.0), respectively |

www.rheumatology.oxfordjournals.org 2259

Downloaded from https://academic.oup.com/rheumatology/article-abstract/54/12/2257/1793409 by guest on 11 March 2018
Based on the findings of power Doppler by US, patients in clinical remission were divided into two groups: USPD(+) and USPD(−). Periodontal pathogens tested in this study were *Aggregatibacter actinomycetemcomitans* (Aa), *Eikenella corrodens* (Ec), *Porphyromonas gingivalis* (Pg) and *Prevotella intermedia* (Pi).

**Fig. 1** Antibody titres against periodontal pathogens in active RA patients and patients in clinical remission

**Table 2** Clinical and serological data from subjects with active RA or RA in clinical remission

<table>
<thead>
<tr>
<th>Variable</th>
<th>RA in non-remission</th>
<th>RA in clinical remission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>USPD (+)</td>
<td>USPD (−)</td>
</tr>
<tr>
<td>HAQ</td>
<td>NT</td>
<td>0.00 (0.00-0.34)</td>
</tr>
<tr>
<td>DAS28-CRP</td>
<td>3.42 (2.86-4.35)</td>
<td>1.45 (1.16-1.70)**</td>
</tr>
<tr>
<td>DAS28-ESR</td>
<td>4.77 (4.07-5.55)</td>
<td>2.00 (1.51-2.59)**</td>
</tr>
<tr>
<td>Total GS score</td>
<td>NT</td>
<td>9 (7-14.75)</td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>0.17 (0.40-2.80)</td>
<td>0.07 (0.03-0.13)**</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>37 (21.25-52.25)</td>
<td>10 (5.5-13)**</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>11.1 (10.4-12.75)</td>
<td>13.1 (12.4-13.9)**</td>
</tr>
<tr>
<td>MMP-3, ng/ml</td>
<td>215.8 (106.4-364.0)</td>
<td>53.5 (33.0-81.6)**</td>
</tr>
<tr>
<td>RF, IU/ml</td>
<td>119 (44-699)</td>
<td>17.5 (4-33)**</td>
</tr>
<tr>
<td>ACPA, U/ml</td>
<td>75.2 (10.2-113.5)</td>
<td>32.6 (5.8-163.0)</td>
</tr>
</tbody>
</table>

All values are given as median (interquartile range), unless otherwise stated. P-value by Kruskal-Wallis test, *P < 0.01 vs active RA, **P < 0.001 vs active RA, ***P < 0.05 vs USPD (+), ****P < 0.001 vs USPD (+) by Dunn’s multiple comparison test. USPD (+): positive synovitis by power Doppler synovitis grade; USPD (−): negative synovitis by power Doppler synovitis grade; GS: lateral views in greyscale mode; Hb: haemoglobin; NT: not tested.
IL-6 and TNF-α markers for RA. Disease activity of RA, and that these might be new bio-
titres against Pi and Ec were associated with the
In the present study, we demonstrated that IgG antibody
destruction of RA [39, 40]. There have been very few
of ACPA, which is related to the aetiology and joint
[36]
the progression and severity of periodontal disease,
differences between the extent of periodontal tissues
patients. However, the present study did not reveal any
differences in IgG antibody titre against Pg between
any of the groups. These discrepancies indicate that
multiple periodontal pathogens are involved in the
pathology of RA. In addition, antibiotics such as
sulphadiazine–trimethoprim, or DMARDs such as salazo-
sulphapyridine may also be related to antibody titres
against periodontal pathogens [49].

It has been reported that periodontal disease in RA
patients can easily become aggravated [35]. In the pre-
sent study, periodontal disease assessments revealed no
differences between the extent of periodontal tissue
destruction and RA disease activity. Similar serum anti-
body titres for each of four periodontal pathogens were
observed in patients with fewer remaining teeth and pa-
tients with greater numbers of remaining teeth (data not
shown). It has recently been suggested that periodontal
pathogens are able to migrate to other tissues, such as
the coronary artery and liver via circulation or the digestive
tract [50, 51]. Migration to sites other than periodontal
tissues may affect serum antibody titres of periodontal
pathogens, and further investigation is necessary to con-
firm these possibilities.

The limitations of this study were as follows: first, as
patients with active RA were already receiving treatment

Discussion

We assessed synovitis by musculoskeletal US and measured serum IgG antibody titre against periodontal pathogens in RA patients in clinical remission and non-remission. Musculoskeletal US examination showed that 68.6% of patients with RA in clinical remission had grade \( \geq 1 \) blood-flow pattern and exhibited synovitis. In addition, the serum IgG antibody titre against Ec in the non-remission RA(+) group was significantly greater when compared with the USPD(+) and USPD(−) groups, and the IgG antibody titre against Pi in the non-remission RA and USPD(+) groups was greater than that of the USPD(−) group. These findings suggest that RA is associated with infection by periodontal pathogens.

Musculoskeletal US examination is useful for the diagnosis of RA and evaluation of its activity. It has been reported that synovitis is detected by musculoskeletal US in half of RA patients who have achieved clinical remission [5–8], and our results are consistent with these reports. Moreover, similar to the report by Brown et al. [31], patients with active RA and in clinical remission can be distinguished by serological biomarkers and disease activity scores such as DAS28; however, it has been suggested that the presence of synovitis cannot be predicted. It has been reported that progression of joint destruction may occur when a blood flow of grade \( \geq 2 \) is observed in the synovium by US [31–34]. Therefore, in RA patients in clinical remission, assessment by musculoskeletal US examination is important, in addition to established biomarkers and disease activity indices.

RA patients have a high incidence of periodontal disease, and periodontal disease can be readily aggravated. It has been reported that RA patients with periodontal disease have high arthritis activity [35], and IgG antibody titres against periodontal pathogens have been investigated as new biomarkers for RA. Numerous anaerobic periodontal pathogens have been identified in the periodontal pockets of patients with periodontal disease, and the presence of Aa, Ec, Pg and Pi is implicated in the progression and severity of periodontal disease [36–38]. Pg is considered to be involved in the production of ACPA, which is related to the aetiology and joint destruction of RA [39, 40]. There have been very few reports in which RA has been associated with Pi and Ec. In the present study, we demonstrated that IgG antibody titres against Pi and Ec were associated with the disease activity of RA, and that these might be new biomarkers for RA.

Pi activates macrophages and induces production of IL-6 and TNF-α [41]. These inflammatory cytokines are known to play a role in periodontal destruction in periodontitis [41–43] and have been also reported to be involved in synovitis, as well as in the joint destruction in RA. Serum antibody levels against Pi were significantly higher in RA patients than in the healthy controls [44]. Significantly higher levels of IgG antibodies against Pi were demonstrated in SF from RA patients than in SF from OA patients [45]. Martínez-Martínez et al. [43] and Moen et al. [46] reported that bacterial DNA of Pi was frequently detected in the SF of patients with RA. The present study demonstrated that IgG antibody titre against Pi in the non-remission RA group and the USPD(+) group was greater than that of the USPD(−) group. Thus, it is suggested that Pi is closely related to synovitis in RA, but the causal relationship remains unclear. Furthermore, it is necessary to determine whether the high antibody titre decreases with treatment for RA, or whether treatment for periodontitis could affect the disease activity of RA.

There are opposing opinions regarding the association between Ec or Pg and RA. Okada et al. [47] reported that the antibody titre of Ec in RA patients is lower than that of healthy subjects. In contrast, Ziebolz et al. [48] showed that Fusobacterium nucleatum (98%), Peptostreptococcus micros (88%) and Ec (91%) were frequently present in the periodontal tissues of RA patients with periodontal disease. However, Pg (58%) was reported to be present at a lower frequency. In this study, the IgG antibody titre against Ec was elevated only in non-remission RA patients. The serum IgG antibody titre against Pg is reported to be elevated in RA patients and to subsequently decrease with treatment [47]. Martínez-Martínez et al. [43] reported that bacterial DNA was frequently detected in the serum and SF of RA patients. However, the present study did not reveal any differences in IgG antibody titre against Pg between any of the groups. These discrepancies indicate that multiple periodontal pathogens are involved in the pathology of RA. In addition, antibiotics such as sulphadiazine–trimethoprim, or DMARDs such as salazo-
sulphapyridine may also be related to antibody titres against periodontal pathogens [49].

The limitations of this study were as follows: first, as patients with active RA were already receiving treatment

\( P = 0.51 \) and clinical attachment levels, which are measures of the progression and severity of periodontitis, did not differ between the groups [probing pocket depth: 2.38 (2.08, 2.62), 2.66 (2.31, 3.03) and 2.58 (2.24, 2.36), respectively \( P = 0.23 \); clinical attachment level: 4 (1.5, 5), 4 (1, 5) and 3 (1.5, 5), respectively \( P = 0.73 \)]. One patient in the non-remission RA group and five patients in the USPD(+) group had \( \leq 5 \) teeth remaining.
with medications such as DMARDs, antibiotics and steroids, it is possible that drug treatment affected the serum antibody titres of periodontal pathogens; and second, the results in this study did not include data obtained through repeated measurements in the same subjects at each of the different disease stages. To understand the effects of periodontal pathogens on disease stage, it will be necessary to evaluate long-term treatment effects for each patient and the association with antibody titres. We could be commercially available to investigate only IgG antibodies to periodontal pathogens, but not other classes of antibody. Further studies are needed to evaluate IgA and IgM antibodies.

Acknowledgements

Y.K., S.Y. and T.T. designed the study; T.I. and A.Y. assessed the physical examination of joints; Y.K. and S.Y. conducted the US examination; M.K. assessed the periodontal disease; Y.K., M.K. and S.M. collected and analysed the data; Y.K., M.K., T.T. and Y.T. wrote the manuscript. Y.K., M.K., T.T. and T.H. revised the manuscript.

Funding: No specific funding was received from any funding bodies in the public, commercial or not-for-profit sectors to carry out the work described in this manuscript.

Disclosure statement: The authors have declared no conflicts of interest.

References

11 Havemose-Poulsen A, Serensen LK, Bendtzen K, Holmstrup P. Polymorphisms within the IL-1 gene cluster: effects on cytokine profiles in peripheral blood and whole blood cell cultures of patients with aggressive periodontitis, juvenile idiopathic arthritis, and rheumatoid arthritis. J Periodontol 2007;78:475–92.


