Antibodies against *Porphyromonas gingivalis* in seropositive arthralgia patients do not predict development of rheumatoid arthritis

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Letter to the editor

Clinical studies point towards an association between periodontitis and rheumatoid arthritis (RA) [1, 2]. A pathogenic role is suggested for *Porphyromonas gingivalis* [3]. *P. gingivalis* may contribute to the pathogenesis of RA by breaking immune tolerance through formation of (bacterial and human) citrullinated protein antibodies, leading to anticitrullinated protein antibody production (ACPA) [4, 5]. Since ACPA production precedes RA development [7] and because *P. gingivalis* IgG antibodies are long-term stable in untreated periodontitis patients [8], we investigated whether anti-*P. gingivalis* antibody levels are prognostic for development of RA, by assessing these antibodies in a cohort of 289 adults at risk for RA.

Patients with arthralgia and seropositivity for IgM rheumatoid factor (IgM RF) and/or ACPA were selected from a prospective follow-up study on arthritis development [9]. The occurrence of arthralgia in people with these autoantibodies probably represents a late stage in the preclinical development of (rheumatoid) arthritis, especially if the symptoms are symmetrically located in the small joints, a situation which could be named ‘inflammatory arthralgia’ [10]. They are further referred to as seropositive arthralgia patients (SAP); their median follow-up was 30 months (IQR 13–49).

Baseline sera were used for measurement of ACPA, IgM RF, C-reactive protein (CRP) and HLA-DRB1 SE carrier status [9]. IgA, IgG and IgM antibody levels against *P. gingivalis* were determined by in-house ELISA with a pooled lysate of clinical isolates of *P. gingivalis* as antigen [11]. Interference of IgM RF on anti-*P. gingivalis* antibody assays was excluded by spiking samples with sera with known high titres of RF.

Reference groups for antibody levels against *P. gingivalis* consisted of healthy subjects without periodontitis and without cultivable subgingival *P. gingivalis* (HC, n = 36, mean age 34 ± 15 years, 53% female, 14% current smoker) and severe periodontitis patients without systemic disease (PD, n = 117, mean age 51 ± 9.3 years, 58% female, 43% current smoker, 42% of n = 45 *P. gingivalis*-culture positive [12]. Both groups were recruited among subjects planned for first consultation at the dental department of the University Medical Center Groningen and a referral practice for periodontology (Clinic for Periodontology Groningen) [11].

IgA and IgG anti-*P. gingivalis* were higher in PD than in HC (both p < 0.0001). PD culture-positive for subgingival *P. gingivalis* had higher IgA and IgG anti-*P. gingivalis* than culture-negative PD (p < 0.01 and p < 0.001). No differences were found for IgM anti-*P. gingivalis*.

Cut-off values for anti-*P. gingivalis* positivity were set at >2 SD above the mean of HC. Influence of anti-*P. gingivalis* positivity on RA development was analyzed using a multivariate Cox proportional hazards model with time until RA development as dependent variable and age, gender, HLA-DRB1 SE carriage, smoking, number of tender joints, and CRP-ACPA- and IgM RF-positivity at inclusion as other variables.

After 12 months (median, IQR 6–20), 33% (n = 94) of SAP had developed RA according to 2010 American College of Rheumatology/European League against Rheumatism criteria [13]. Baseline characteristics of SAP who developed RA (RA+) or did not develop RA (RA−) are listed in Table 1, page 50.

In SAP, IgG anti-*P. gingivalis* was higher than in HC, but lower than in PD, as was IgA anti-*P. gingivalis* (Fig. 1A, page 51). No differences in IgM anti-*P. gingivalis* were found, nor were differences found for anti-*P. gingivalis* antibody levels between ACPA-positive or ACPA-negative SAP.

SAP who developed RA did not have elevated anti-*P. gingivalis* antibody levels at baseline compared with SAP who did not develop RA.
within the follow-up period (Fig. 1B, page 51). When using cut-off values for anti-\textit{P. gingivalis} positivity, the proportion of IgA and IgG anti-\textit{P. gingivalis}-positive patients was higher in SAP who did not develop RA (Table 1, page 50). Besides a weak correlation of IgM anti-\textit{P. gingivalis} with ACPA in SAP who developed RA ($p < 0.05, \rho = 0.23$), no other correlation with anti-\textit{P. gingivalis} was found.

The multivariate Cox proportional hazards model showed significant influence of ACPA (HR 11, 95% CI 5.1 to 24, $p < 0.0001$), IgM RF (HR 2.5, 95% CI 1.6 to 4.1, $p < 0.0001$), number of tender joints (HR 1.05, 95% CI 1.01 to 1.09, $p < 0.05$) and HLA-DRB1 SE carriage (HR 1.7, 95% CI 1.1 to 2.6, $p < 0.05$) on RA development. Influence of anti-\textit{P. gingivalis}, CRP, age, gender and smoking could not be established. Within the limitations of this study, we conclude that anti-\textit{P. gingivalis} antibody levels are not prognostic for development of RA.

References


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**Tables and Figures**

**Table 1** Baseline characteristics of seropositive arthralgia patients (SAP) who did (RA+) or did not (RA−) develop rheumatoid arthritis within the follow-up period.

<table>
<thead>
<tr>
<th></th>
<th>All SAP</th>
<th>RA+</th>
<th>RA−</th>
<th>P value RA+ vs. RA−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>289</td>
<td>94</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>Female, percentage</td>
<td>79</td>
<td>81</td>
<td>78</td>
<td>0.76</td>
</tr>
<tr>
<td>Mean age in years (SD)</td>
<td>50 (12)</td>
<td>48 (11)</td>
<td>50 (12)</td>
<td>0.19</td>
</tr>
<tr>
<td>Smoking at inclusion, percentage</td>
<td>29</td>
<td>35</td>
<td>26</td>
<td>0.13</td>
</tr>
<tr>
<td>HLA-DRB1 SE, percentage</td>
<td>40</td>
<td>45</td>
<td>37</td>
<td>0.19</td>
</tr>
<tr>
<td>Seropositive for IgM-RF, percentage</td>
<td>61</td>
<td>57</td>
<td>63</td>
<td>0.37</td>
</tr>
<tr>
<td>Seropositive for IgG ACPA, percentage</td>
<td>65</td>
<td>90</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>Median (IQR) hsCRP (mg/L)</td>
<td>2.2 (1.0-4.8)</td>
<td>2.6 (1.0-4.6)</td>
<td>2.0 (0.9-5.1)</td>
<td>0.47</td>
</tr>
<tr>
<td>Median (IQR) TJC53 at inclusion</td>
<td>0 (0-3)</td>
<td>1 (0-4)</td>
<td>0 (0-3)</td>
<td>0.1</td>
</tr>
<tr>
<td>Median (IQR) follow-up in months</td>
<td>30 (13-49)</td>
<td>25 (12-46)</td>
<td>34 (15-49)</td>
<td>0.05</td>
</tr>
<tr>
<td>Median (IQR) time until RA development</td>
<td>12 (6-20)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive for anti- <em>P. gingivalis</em> IgA, percentage†</td>
<td>20</td>
<td>11</td>
<td>25</td>
<td>0.01</td>
</tr>
<tr>
<td>Positive for anti- <em>P. gingivalis</em> IgG, percentage†</td>
<td>34</td>
<td>26</td>
<td>37</td>
<td>0.05</td>
</tr>
<tr>
<td>Positive for anti- <em>P. gingivalis</em> IgM, percentage†</td>
<td>6.9</td>
<td>5.3</td>
<td>7.7</td>
<td>0.62</td>
</tr>
</tbody>
</table>

*Variables reflected in percentages: Fisher’s exact test with two sided p value, other variables: unpaired t-test with Welch’s correction (Gaussian distribution) or Mann–Whitney U test (no Gaussian distribution).

†Positivity is defined as >2 SD above the mean anti-*P. gingivalis* levels of healthy controls.

ACPA: anti-citrullinated protein antibodies, cut off level for positivity 5 U/mL, HLA-DRB1 SE: one or two copies of the HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410 or *1001 alleles, hsCRP: high-sensitivity C-reactive protein, RA: rheumatoid arthritis, RF: rheumatoid factor, cut off level for positivity 30 IU/mL, TJC53: tender joint count 53 joints.
Fig. 1 (A) IgA, IgG and IgM anti-\textit{Porphyromonas gingivalis} antibody levels in seropositive arthralgia patients (SAP) compared with severe periodontitis patients without other systemic disease and healthy controls with a healthy periodontium and no cultivable subgingival \textit{P. gingivalis} (HC). (B) IgA, IgG and IgM anti-\textit{P. gingivalis} antibody levels in SAP who developed rheumatoid arthritis (RA+) and SAP who did not develop rheumatoid arthritis (RA−) according to the 2010 American College of Rheumatology (ACR)/European League against Rheumatism (EULAR) criteria.

Solid lines represent median values. Dotted lines indicate arbitrary cut-off values for anti-\textit{P. gingivalis} positivity defined as >2 SD above the mean of the healthy controls. Comparison of three groups: Kruskal–Wallis one-way analysis of variance with Dunn’s multiple comparison post-test if overall p < 0.05. Comparison of two groups: Mann–Whitney U test with two-sided p value. *p < 0.05, **p < 0.001.

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