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

Clinical studies point towards an association between periodontitis and rheumatoid arthritis (RA). A pathogenic role is suggested for Porphyromonas gingivalis. P gingivalis may contribute to the pathogenesis of RA by breaking immune tolerance through formation of (bacterial and human) citrullinated proteins, leading to anticitrullinated protein antibody production (ACPA). Since ACPA production precedes RA development and because P gingivalis IgG antibodies are long-term stable in untreated periodontitis patients, 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. 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. 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. 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 ±13 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% P gingivalis-culture positive). 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).

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 mean +2 SD of HC. Influence of anti-P gingivalis positivity on RA development was analysed using a multivariate Cox proportional hazards analysis 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. Baseline characteristics of SAP who developed RA (RA+) or did not develop RA (RA−) are listed in table 1.

In SAP IgG anti-P gingivalis was higher than in HC, but lower than in PD, as was IgA anti-P gingivalis (figure 1A). 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 (figure 1B). When using cut-off values for anti-P gingivalis positivity, the proportion of IgA and IgG anti-P gingivalis-positive patients was higher in SAP who did not develop RA (table 1). Besides a weak correlation of IgM anti-P gingivalis with ACPA in SAP who developed RA (p<0.03, r=0.23), no other correlation with anti-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. In influences of anti-P gingivalis, CRP, age, gender and smoking could not be established. Within the limitations of this study, we conclude that anti-P gingivalis antibody levels are not prognostic for development of RA.

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Figure 1 (A) IgA, IgG and IgM anti-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 P gingivalis (HC). (B) IgA, IgG and IgM anti-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-P gingivalis positivity defined as mean values plus 2 SDs 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 test with two-sided p value. *p<0.05, **p<0.001.

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>N</td>
<td>289</td>
<td>94</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>Female (%)</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 (%)</td>
<td>29</td>
<td>35</td>
<td>26</td>
<td>0.13</td>
</tr>
<tr>
<td>HLA-DRB1 SE (%)</td>
<td>40</td>
<td>45</td>
<td>37</td>
<td>0.19</td>
</tr>
<tr>
<td>Seropositive for IgM-RF (%)</td>
<td>61</td>
<td>57</td>
<td>63</td>
<td>0.37</td>
</tr>
<tr>
<td>Seropositive for IgG ACPA (%)</td>
<td>65</td>
<td>90</td>
<td>53</td>
<td>0.00</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>0.10</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>–</td>
<td>11 (6–20)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Positive for anti-Porphyromonas gingivalis IgA (%)†</td>
<td>20</td>
<td>11</td>
<td>25</td>
<td>0.01</td>
</tr>
<tr>
<td>Positive for anti-P gingivalis IgG (%)†</td>
<td>34</td>
<td>26</td>
<td>37</td>
<td>0.05</td>
</tr>
<tr>
<td>Positive for anti-P gingivalis IgM (%)†</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 test (no Gaussian distribution).
†Positivity is defined as higher than mean+2SD of anti-P gingivalis levels of healthy controls.
ACPA, anticitrullinated 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; N, number; RA, rheumatoid arthritis; RF, rheumatoid factor, cut-off level for positivity 30 IU/mL; TJC53, tender joint count 53 joints.
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Competing interests None.

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