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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).^{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 proteins, leading to anticitrullinated protein antibody production (ACPA).^{4,5} Since ACPA production precedes RA development⁶ and because *P. gingivalis* IgG antibodies are long-term stable in untreated periodontitis patients,⁷ 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.⁸ 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.⁸ 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.⁹ 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% *P. gingivalis*-culture positive).¹⁰ 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).⁹

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 ($p < 0.05$, $r = 0.23$), 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, $p < 0.0001$),

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

	All SAP	RA+	RA−	p Value RA+ vs RA−*
N	289	94	195	
Female (%)	79	81	78	0.76
Mean age in years (SD)	50 (12)	48 (11)	50 (12)	0.19
Smoking at inclusion (%)	29	35	26	0.13
HLA-DRB1 SE (%)	40	45	37	0.19
Seropositive for IgM-RF (%)	61	57	63	0.37
Seropositive for IgG ACPA (%)	65	90	53	0.00
Median (IQR) hsCRP (mg/L)	2.2 (1.0–4.8)	2.6 (1.0–4.6)	2.0 (0.9–5.1)	0.47
Median (IQR) TJC53 at inclusion	0 (0–3)	1 (0–4)	0 (0–3)	0.10
Median (IQR) follow-up in months	30 (13–49)	25 (12–46)	34 (15–49)	0.05
Median (IQR) time until RA development	—	12 (6–20)	—	—
Positive for anti- <i>Porphyromonas gingivalis</i> IgA (%)†	20	11	25	0.01
Positive for anti- <i>P. gingivalis</i> IgG (%)†	34	26	37	0.05
Positive for anti- <i>P. gingivalis</i> IgM (%)†	6.9	5.3	7.7	0.62

*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.

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. * $p < 0.05$, ** $p < 0.001$.

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. 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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Contributors Study conception and design: JW, EB, LAvdS and DvS. Acquisition of data: LAvdS, DvS, MdS, BD-vdM and KMJJ. Analysis and interpretation of data: MdS, KMJJ, EB, AJvW, AV, JW and DvS. Drafting of manuscript: MdS. Critical revision: AJvW, AV, EB, JW and DvS.

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Competing interests None.

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