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

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Published in:
Annals of the Rheumatic Diseases

DOI:
10.1136/annrheumdis-2013-204594

Publication date:
2014

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 development[6] 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-*Porphyromonas gingivalis* IgA (%)†	20	11	25	0.01
Positive for anti-*P gingivalis* IgG (%)†	34	26	37	0.05
Positive for anti-*P gingivalis* 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.

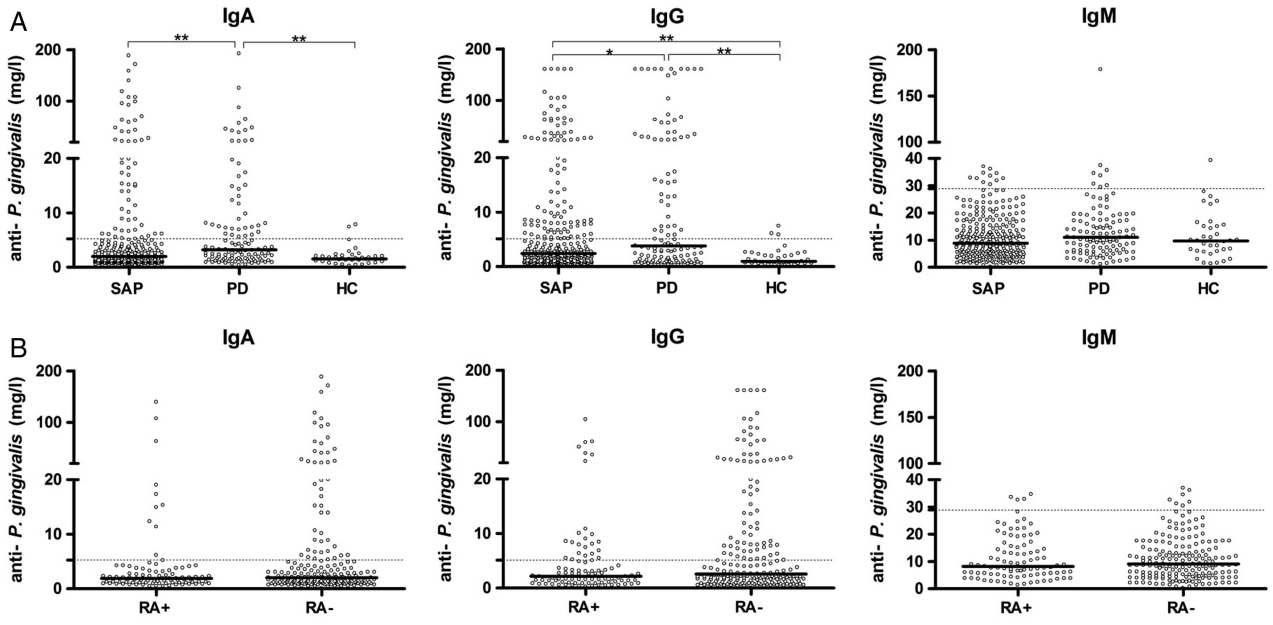

Figure 1 (A) IgA, IgG and IgM anti-*Porphyromonas gingivalis* antibody levels in seropositive arthralgia patients (SAP) compared with severe periodontitis patients without other systemic disease and healthy controls with a healthy periodontium and no cultivable subgingival *P gingivalis* (HC). (B) IgA, 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.

Funding This study was funded by the authors' institutions. The SAP cohort was supported by grants from the Netherlands Organization for Health Research and Development (ZonMw) (grant number 6100.0010) and the Dutch Arthritis Association (grant number 0801034).

Competing interests None.

Ethics approval Ethics Committee of the Slotervaart Hospital and the Jan van Breemen Institute, Amsterdam, The Netherlands.

Provenance and peer review Not commissioned; externally peer reviewed.

▶ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2013-204594).

To cite de Smit M, van de Stadt LA, Janssen KMJ, *et al*. *Ann Rheum Dis* 2014;**73**:1277–1279.

Accepted 21 December 2013
Published Online First 13 January 2014

Ann Rheum Dis 2014;**73**:1277–1279. doi:10.1136/annrheumdis-2013-204594

REFERENCES

1 de Pablo P, Chapple IL, Buckley CD, *et al*. Periodontitis in systemic rheumatic diseases. *Nat Rev Rheumatol* 2009;5:218–24.
2 Janssen KM, Vissink A, de Smit MJ, *et al*. Lessons to be learned from periodontitis. *Curr Opin Rheumatol* 2013;25:241–7.
3 Rosenstein ED, Greenwald RA, Kushner LJ, *et al*. Hypothesis: the humoral immune response to oral bacteria provides a stimulus for the development of rheumatoid arthritis. *Inflammation* 2004;28:311–18.
4 Quirke AM, Lugli EB, Wegner N, *et al*. Heightened immune response to autocitrullinated *Porphyromonas gingivalis* peptidylarginine deiminase: a potential mechanism for breaching immunologic tolerance in rheumatoid arthritis. *Ann Rheum Dis* 2014;73:263–9.
5 Wegner N, Wait R, Sroka A, *et al*. Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and alpha-enolase: implications for autoimmunity in rheumatoid arthritis. *Arthritis Rheum* 2010;62:2662–72.
6 Nielen MM, van SD, Reesink HW, *et al*. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004;50:380–6.
7 Lakio L, Antinheimo J, Paju S, *et al*. Tracking of plasma antibodies against Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis during 15 years. *J Oral Microbiol* 2009. doi:10.3402/jom.v1i0.1979
8 van de Stadt LA, van der Horst AR, de Koning MH, *et al*. The extent of the anti-citrullinated protein antibody repertoire is associated with arthritis development in patients with seropositive arthralgia. *Ann Rheum Dis* 2011;70:128–33.
9 de Smit M, Westra J, Vissink A, *et al*. Periodontitis in established rheumatoid arthritis patients: a cross-sectional clinical, microbiological and serological study. *Arthritis Res Ther* 2012;14:R222.
10 Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999;4:1–6.