Periodontitis and rheumatoid arthritis

de Smit, Menke

CHAPTER

03

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

Menke de Smit[1], Lotte Arwen van de Stadt[2,3], Koen Janssen[4], Berber Doornbos-van der Meer[5], Arjan Vissink[4], Arie Jan van Winkelhoff[1, 6], Elisabeth Brouwer[5], Johanna Westra[5] and Dirkjan van Schaardenburg[3, 7]

[1]Center for Dentistry and Oral Hygiene, University of Groningen, University Medical, Center Groningen, Groningen, The Netherlands

[2]Sanquin Research and Landsteiner Laboratory, Academic Medical Center, Amsterdam, The Netherlands

[3]Jan van Breemen Research Institute | Reade, Amsterdam, The Netherlands

[4]Department of Oral and Maxillofacial Surgery, University of Groningen, University, Medical Center Groningen, Groningen, The Netherlands

[5]Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

[6]Department of Medical Microbiology, Center for Dentistry and Oral Hygiene, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

[7]Department of Rheumatology, VU University Medical Center, Amsterdam, The Netherlands

Annals of the Rheumatic Diseases 2014 Jun;73(6):1277-9. doi: 10.1136/annrheumdis-2013-204594. Epub 2014 Jan 13.

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 proteins, 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-*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, page 50). Besides a weak correlation of IgM anti-*P. gingivalis* with ACPA in SAP who developed RA ($p < 0.05$, $\rho = 0.23$), 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. Influence 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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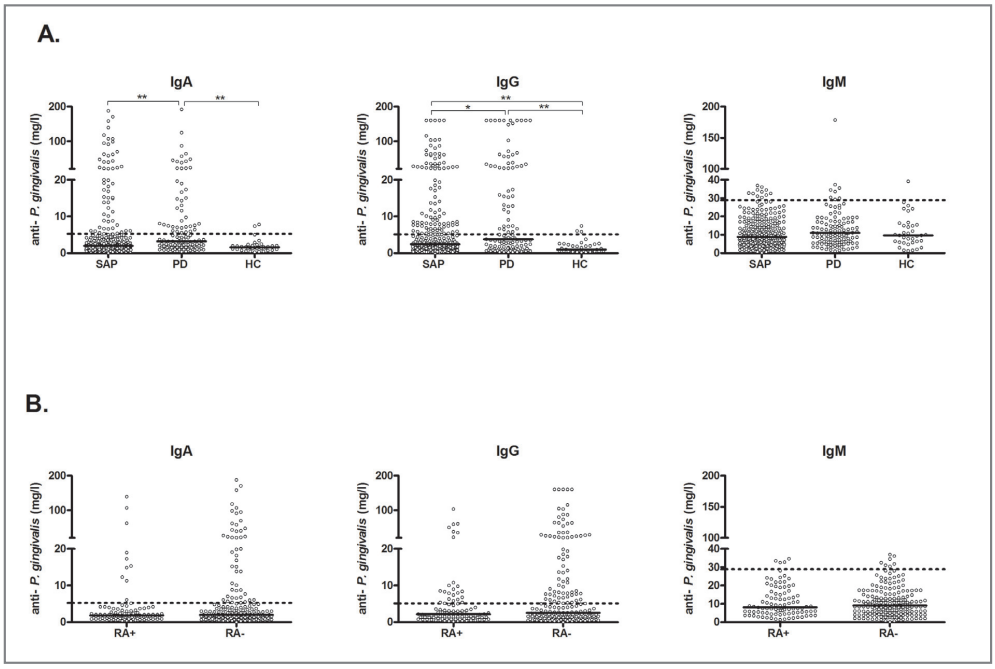
Tables and Figures

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

	All SAP	RA+	RA-	*P* value RA+ vs. RA-
Number	289	94	195	
Female, percentage	79	81	78	0.76
Mean age in years (SD)	50 (12)	48 (11)	50 (12)	0.19
Smoking at inclusion, percentage	29	35	26	0.13
HLA-DRB1 SE, percentage	40	45	37	0.19
Seropositive for IgM-RF, percentage	61	57	63	0.37
Seropositive for IgG ACPA, percentage	65	90	53	0
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)	0.1
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- *P. gingivalis* IgA, percentage†	20	11	25	0.01
Positive for anti- *P. gingivalis* IgG, percentage†	34	26	37	0.05
Positive for anti- *P. gingivalis* IgM, percentage†	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 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-*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 >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.*