

Increased IgA anti-citrullinated protein antibodies in the periodontal inflammatory exudate of healthy individuals compared to rheumatoid arthritis patients

Poerwati Soetji Rahajoe¹ | Menke de Smit^{2,3}  | Gerbrich Schuurmans³ |
Elisabeth Raveling-Eelsing³ | Nyoman Kertia⁴ | Arjan Vissink²  | Johanna Westra³ 

¹Department of Oral Surgery, Dr. Sardjito General Hospital, Yogyakarta, Indonesia

²Department of Oral Surgery, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

³Department of Rheumatology and Clinical Immunology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

⁴Department of Rheumatology, Dr. Sardjito General Hospital, Yogyakarta, Indonesia

Correspondence

Menke de Smit, Department of Oral and Maxillofacial Surgery, University of Groningen, University Medical Center Groningen, Hanzeplein 1, Postbus 30.001, 9700 RB Groningen, The Netherlands.
Email: m.j.de.smit@umcg.nl

Funding information

This research was partly funded by the Dutch Arthritis Foundation (ReumaNederland, grant number 16-2-201) as well as by a Abel Tasman Talent Program sandwich PhD grant from the Graduate School of Medical Sciences of the University of Groningen.

Abstract

Aim: To assess rheumatoid arthritis (RA)-associated autoantibodies in the gingivocrevicular fluid (GCF) of RA patients and healthy controls with or without periodontal disease, as chronic mucosal inflammation in periodontal disease is hypothesized to contribute to the formation of these autoantibodies.

Materials and methods: Anti-citrullinated protein antibodies (ACPA), rheumatoid factor (RF), and their IgA isotypes were assessed in the serum and GCF of RA patients ($n = 72$) and healthy controls (HC, $n = 151$). The presence and levels of these antibodies were studied in relation to interleukin (IL)-8 and periodontal disease.

Results: In contrast to the HC, the levels of ACPA and RF in the serum and GCF of the RA patients were strongly correlated ($p < .0001$). The HC with high levels of IgA-ACPA ($n = 27$) also had significantly higher levels of total IgG, total IgA, and IL-8 in the GCF than the HC with low levels of IgA-ACPA in the GCF ($n = 124$). Periodontal inflammation and smoking were seen more frequently in the group with high levels of IgA-ACPA compared to the group with low IgA-ACPA.

Conclusion: The IgA-ACPA in the GCF of HC may be associated with periodontal inflammation and smoking, and could be involved in the progression to RA.

KEYWORDS

anti-citrullinated protein autoantibodies, mucosal inflammation, rheumatoid arthritis, rheumatoid factor

1 | INTRODUCTION

Antibodies against citrullinated proteins (ACPA) and IgG-Fc (rheumatoid factor, RF) are a hallmark of rheumatoid arthritis (RA). Seropositivity for these autoantibodies can be detected years before

clinical disease onset (Malmström, Catrina, & Klareskog, 2017), and the presence of ACPA in RA is associated with worse disease outcome (van der Helm-van Mil, Verpoort, Breedveld, Toes, & Huizinga, 2005). The aetiology of immune dysregulation and autoimmunity in RA is still unclear, but is presumed to be initiated at inflamed mucosal

Poerwati Soetji Rahajoe and Menke de Smit contributed equally.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2020 The Authors. *Journal of Clinical Periodontology* published by John Wiley & Sons Ltd.

surfaces, specifically the mucosal surfaces of the lungs and oral cavity (i.e. the periodontium), in combination with genetic and environmental factors such as smoking (Mikuls, Payne, Deane, & Thiele, 2016).

Periodontal disease is primarily triggered by bacterial infection and leads to inflammation and destruction of the periodontium. Smoking leads to increased susceptibility to, greater severity and faster progression of periodontal disease (Nociti, Casati, & Duarte, 2015). Besides local tissue destruction, the continuous low-grade inflammation may, partly because of hyperreactive neutrophils (Ling, Chapple, & Matthews, 2015), have systemic consequences. The established bidirectional association between the existence and/or severity of periodontal disease and systemic (autoimmune) diseases such as RA (Kumar, 2016; Smit et al., 2012) can be explained by, amongst others, shared risk factors like genetic predisposition and smoking, a higher local and systemic inflammatory burden as well as by the induction of autoimmunity by chronic inflammation (Potempa, Mydel, & Koziel, 2017). However, ACPA seropositivity was found to be rather low in patients with periodontal disease without RA (de Pablo et al., 2014; Janssen et al., 2015), whereas their RF seropositivity was slightly higher (Janssen et al., 2015; Thé & Ebersole, 1991).

Citrullination is catalysed by endogenous peptidyl arginine deiminase (PAD) enzymes in neutrophils and monocytes, and is thought to play an important role in RA. PAD is also essential for antibacterial innate immunity mediated by forming neutrophil extracellular traps (NETs), in the form of highly decondensed chromatin structures. Histone hypercitrullination catalysed by PAD4 correlates with chromatin decondensation during NET formation (Li et al., 2010). NETs have been implicated in a number of autoimmune conditions, including RA, but also in periodontal disease. As PAD enzymes are not only necessary for NET formation but also for ACPA formation, it is interesting to hypothesize that their activity may link the two diseases together (Cooper, Palmer, & Chapple, 2013). Another potential mechanistic link between periodontal disease and RA comes from the discovery that the periodontal pathogen *Porphyromonas gingivalis* has the unique ability to express its own PAD enzyme (PPAD), which led to the hypothesis that the breakdown in the tolerance to citrullinated proteins is initiated by PPAD dependent citrullination (Rosenstein, Greenwald, Kushner, & Weissmann, 2004; Wegner et al., 2010). An *Aggregatibacter actinomycetemcomitans* periodontal infection could be another mechanistic link to initiating autoimmunity in RA. *Aggregatibacter actinomycetemcomitans* leukotoxin (LtxA) is able to dysregulate the activation of PAD enzymes in host neutrophils leading to the release of hypercitrullinated proteins, thereby mimicking the repertoire of citrullinated antigens found in the RA joint (Konig et al., 2016).

Gingival crevicular fluid (GCF), the periodontal exudate, can be collected from the gingival crevice around the teeth. It is composed of serum and locally generated components. Leakage of GCF increases when the gingival crevice becomes inflamed (Champagne et al., 2003). Although it is hypothesized that a chronically inflamed periodontium is a potential site at which immune tolerance to citrullinated epitopes is broken and the production of ACPA begins, the

Clinical Relevance

Scientific rationale for the study: Mucosal inflammation in periodontal disease has been hypothesized to contribute to autoimmunity in RA. Arthritis autoantibodies can be present years before clinical disease presentation, and there is ample evidence that these autoantibodies are present in the periodontium.

Principal findings: In contrast to the RA patients, arthritis autoantibodies were present in the GCF but not in the serum of the healthy controls, implicating local induction of these autoantibodies. In the healthy controls, this was related to periodontal inflammation and smoking.

Practical implications: Mucosal inflammation, due to periodontal disease and smoking, could be involved in RA development.

presence of ACPA in GCF has hardly been investigated even though older studies show that RF is present and produced in inflamed periodontal tissue (Gargiulo, Robinson, Toto, & Gargiulo, 1982; Hirsch, Tarkowski, Koopman, & Mestecky, 1989). We know that inflamed periodontal tissue as well as the GCF of periodontally inflamed sites contains citrullinated peptides (Hendler et al., 2010; Nesse et al., 2012; Schwenger et al., 2017). Tissue expression of citrullinated proteins and the expression of PAD2 and 4, which are important in RA, increase with inflammation severity (Harvey et al., 2013). In addition, the activity of PAD and PPAD in GCF increases in the presence of periodontal disease (Laugisch et al., 2016). Up to now, only Harvey et al. (2013) demonstrated the presence of ACPA in the GCF of some patients with periodontal disease. There are no reports of ACPA, RF or other RA-associated autoantibodies in RA patients' GCF (Rahajoe, de Smit, Kertia, Westra, & Vissink, 2019). Therefore, the aim of this study was to assess RA-associated autoantibodies, especially the IgA isotypes of ACPA and RF because they are specific for mucosal inflammation in the GCF of RA patients and in healthy controls with or without periodontal disease. The presence of these RA-associated autoantibodies is related to the total periodontal inflamed surface area (PISA; Nesse et al., 2008), as well as to the local presence of the chemokine interleukin-8, as a chemoattractant for neutrophils, important for inducing NET formation (Gonzalez-Aparicio & Alfaro, 2019).

2 | MATERIALS AND METHODS

Rheumatoid arthritis patients ($n = 72$) were consecutively recruited at the outpatient Rheumatology Clinic, Sardjito Hospital, University of Gadjah Mada in Yogyakarta, Indonesia between June 2014 and June 2015. The inclusion criteria were fulfilling the ACR (American College of Rheumatology) classification criteria for RA 2010 (Aletaha et al., 2010). The exclusion criteria were as

follows: under 18 years of age, edentulism, diabetes, cardiovascular disease with anti-coagulant medication, presence of non-oral infection, antibiotic use <3 months prior the study, presence of malignancy, and pregnancy including a 6-month post-partum period as well as breastfeeding. Consecutive healthy controls (HC, with and without periodontal disease; $n = 151$) were selected from the patients with an appointment for a first consultation for third molar assessment or tooth extraction at the Oral and Maxillofacial Surgery Clinic, Sardjito Hospital or Dental hospital, Gadjah Mada University in Yogyakarta, Indonesia. Inclusion criteria were not suffering from RA and the absence of distinct oral pathology other than periodontal disease (such as cysts, abscesses not due to periodontal disease and a tumour). The same exclusion criteria applied as for the RA patients. This study was reviewed and approved by the Medical and Health Research Ethics Committee of the Medical Faculty of Gadjah Mada University, Yogyakarta, Indonesia according to the Declaration of Helsinki 2008 (Ref: KE/FK/430/EC). All the patients were informed verbally and by letter, and the participants provided written informed consent. We aimed for a larger recruitment (1:2) of the control group to allow for a more reliable comparison and to be able to correct for potential confounders.

2.1 | Clinical examination

The RA patients were clinically examined by a rheumatologist (DW and KN) during their routine visit at the outpatient rheumatology clinic. RA disease activity was measured using the Disease Activity Score 28 joint count (DAS28-ESR; Prevoo et al., 1995). The other recorded parameters included RA disease duration, smoking status (current, former or never), and RA medication. After inclusion, both the RA patients and healthy controls underwent clinical periodontal examination by trained and calibrated dentists (MT and AM). Full-mouth oral measurements at 6 sites per tooth (pocket probing depth, bleeding on probing, and periodontal attachment loss) were recorded to calculate the PISA. PISA reflects the extent of inflammatory burden due to periodontal inflammation (Nesse et al., 2008). A PISA value $\geq 130 \text{ mm}^2$ has been shown to be associated with the Centers for Disease Control and Prevention—American Academy of Periodontology (CDC-AAP) case definition of periodontitis (Leira, Martín-Lancharro, & Blanco, 2018).

2.2 | Sampling

Venous blood was collected from all the participants on the day of their (periodontal) examination, and the erythrocyte sedimentation rate (ESR) was measured using the Wintrobe Method. After centrifugation, the serum was stored at -20°C until further analysis. GCF samples were taken from the deepest non-bleeding site per quadrant based on the pocket probing depth measurements. The sample sites were isolated with cotton rolls, and supragingival plaque was carefully removed with curettes and cotton pallets. Subsequently,

two sterile paper points (Inline, BM Dentale) were inserted per site and left in for 10 s. Any GCF samples visibly contaminated with blood were discarded. The GCF samples were pooled per patient, wrapped in aluminium foil and stored in a sterile sample cup (1.5 ml, Stardec, TG Medical) at -20°C . The GCF analysis involved eluting the paper points overnight at 4°C in 200 μl phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA), using three paper points per elution. After centrifugation at 1,550 g for 20 min, the paper points were removed and the supernatants were stored at -20°C until further analysis.

2.3 | Laboratory procedures

Total IgG and IgA in the GCF were determined by ELISA using the following protocol: 96 well plates (Corning® Costar®, Sigma-Aldrich) were coated with donkey F(ab)₂ anti-human IgG (1:5,000; Jackson) or mouse anti-human IgA (1:1,500; Temecula) and incubated overnight at room temperature (RT). Standard curves were constructed for both isotypes using the Siemens N protein standard SL starting at 50 ng/ml for IgG and 300 ng/ml for IgA. Fourfold dilutions were made of the IgG and the IgA from the GCF, starting at 1:100 for IgG and 1:5 for IgA and were incubated for 1.5 hr at RT. Mouse anti-human IgG-HRP (1:2,000) and goat anti-human IgA-HRP (1:4,000; both Southern Biotech) conjugates were added to the dilutions for 1 hr at RT. A colour reaction was established using tetramethylbenzidine (Sigma-Aldrich) and hydrogen peroxide.

IgG anti-cyclic citrullinated protein antibody (anti-CCP) levels were measured using a commercial anti-CCP2 kit (Euro Diagnostica) according to the manufacturer's protocol, with a diagnostic cut-off positivity value of $>10 \text{ U/ml}$. Serum samples with a value $<10 \text{ U/ml}$ were measured again with an adjusted protocol whereby the samples were diluted 1:10 instead of 1:50. IgA anti-CCP was measured using a modified anti-CCP2 kit (Janssen et al., 2015) with a goat anti-human IgA-HRP (Southern Biotech)—conjugate dilution of 1:2,000. IgA seropositivity was defined as $>2 \text{ SD}$ above the mean of the healthy controls without periodontal disease and who had never smoked ($n = 88$). Both the IgG and IgA isotypes from the GCF samples were diluted 1:10.

The IgM and IgA RFs were determined in the serum and GCF using an in-house validated ELISA (Van Leeuwen, Westra, van Riel, Limburg, & Van Rijswijk, 1995), with minor modifications: 96 well plates (Corning® Costar®, Sigma-Aldrich) were coated with 40 $\mu\text{g/ml}$ heat-aggregated human IgG diluted in 0.1 M carbonate buffer and incubated overnight at RT. The GCF samples were diluted 2:1 in the plate and incubated for 1 hr at RT. Goat anti-human IgM-HRP or IgA-HRP (both Southern Biotech) was added for 30 min at RT. A colour reaction was established using tetramethylbenzidine and hydrogen peroxide. The levels were expressed in international units (IU) per millilitre, and seropositivity was defined as $>5 \text{ IU/ml}$ for IgM RF and $>25 \text{ IU/ml}$ for IgA RF. Absorbance was read at 450 nm in a VersaMax microplate reader and antibody levels were calculated using the SoftMax PRO software (both Molecular Devices).

TABLE 1 Patient characteristics

Patient characteristics	RA (n = 72)	HC (n = 151)	p value
Age (median, IQR)	48 (40–55)	45 (34–53)	.0583
Female (%) (n)	89 (64)	47 (71)	<.0001
Smokers (%) (n)	9.7 (7)	26 (39)	.0047
Co-morbidity (%) (n)			
Asthma (+bronchodilator)	0 (0)	4.0 (6)	.1805
Hypertension (+medication)	9.7 (7)	4.6 (7)	.1515
Medication for hypertension, also combinations (n)			
Calcium channel blocker	5	5	
Ace inhibitor	2	2	
Angiotensin receptor blocker	1	0	
RA characteristics			
Disease duration in months (median, IQR)	24 (6–48)		
DAS28 (median, IQR)	5.3 (4.2–5.8)		
Disease remission ^a (DAS28 < 2.6) (%) (n)	0 (0)		
Low disease activity (DAS28 ≥ 2.6 < 3.2) (%) (n)	4.2 (3)		
Moderate disease activity (DAS28 ≥ 3.2 < 5.1) (%) (n)	36 (26)		
High disease activity (DAS28 ≥ 5.1) (%) (n)	60 (43)		
ACPA seropositive (%) (n)			
IgG (>10 U/ml)	40 (29)	1.3 (2)	<.0001
IgA ^b (>1.2 U/ml)	39 (28)	6.0 (9)	<.0001
RF seropositive (%)			
IgM (>5 IU/ml)	47 (34)	2.6 (4)	<.0001
IgA (>25 IU/ml)	35 (25)	0 (0)	<.0001
Medication for RA (%) (n), also combinations			
No/herbal	14 (10)		
NSAID	63 (45)		
Steroid	78 (56)		
DMARDS total	57 (41)		
DMARDS specific			
MTX	46 (33)		
Leflunomide	2.8 (2)		
Chloroquine	2.8 (2)		
Sulfasalazine	17 (12)		

^aAccording to (Wells et al., 2009).

^b>2 SD above the mean of non-smoker healthy controls without periodontal disease, n = 88.

IL-8 in the GCF was measured by a DuoSet ELISA (DY208, R&Dsystems, Bio-Techne) according to the manufacturers' instructions. The GCF samples were measured in 1:5 and 1:25 dilutions.

2.4 | Statistics

Statistical analyses were performed using the GraphPad Prism software (version 8 for Windows; GraphPad software). Data were non-normally distributed (based on QQ plots). Regarding group comparisons, a Mann-Whitney *U* test was used for continuous variables and Fisher's exact test for categorical variables. Since the RA patients and HC were not directly matched, it was not necessary to take the unequal group size into account in the statistical comparison at group level. Correlations between different parameters were assessed by Spearman's ρ .

3 | RESULTS

Seventy-two RA patients and 151 HC were analysed. The patient characteristics are listed in Tables 1 and 2. The RA patients comprised more women and fewer smokers than the HC. The median number of teeth was high in both groups (median: 26 for RA and 28 for HC); however, the overall number of teeth in the RA patients was slightly lower. Although the median RA disease activity was high (DAS28 5.3), the seropositivity for ACPA and RF in the RA patients was rather low (40% for IgG ACPA, 39% for IgA-ACPA, 47% for IgM RF, and 35% for IgA RF). Seropositivity in the HCs was significantly lower (1.3% for IgG ACPA, 6.0% for IgA-ACPA, 2.6% for IgM RF, and 0% for IgA RF). PISA in The HC and RAs was not significantly different. There were no differences in the prevalence of periodontal disease between the groups (Table 2). DAS28 was negatively correlated with the number of teeth ($p = .017$).

The ACPA or RF levels in the RA patients' serum were not related to the presence of periodontal disease. Although the overall levels of these autoantibodies were low in the HC serum, IgA-ACPA was slightly higher in those with periodontal disease ($p = .042$; Figure 1a).

Anti-citrullinated protein antibodies and RF were present in the GCF (Figure 1b). The IgA-ACPA levels were significantly higher in the GCF of the HC ($p = .0043$), while the RA patients had significantly higher IgG ACPA and IgM RF levels in the GCF (both $p < .0001$). The IgA RF levels were slightly higher in the RA patients' GCF ($p = .0207$). Total IgG and total IgA in the GCF had increased significantly in the groups with periodontal disease compared to the groups without periodontal disease (RA: $p < .0002$ for IgG and $p < .0073$ for IgA, HC: $p < .0001$ for IgG, $p < .0005$ for IgA), while only the HC periodontal disease group had higher levels of IL-8 ($p = .0204$; Figure 2).

In this study, we found higher levels of IgA-ACPA in the GCF of the HC, whereas the IgA RF levels were comparable among the groups. When calculating the ratios of IgA-ACPA and total IgA as well as IgA RF and total IgA in the GCF, we found that the RA patients' average ACPA/IgA ratio was 1.089 U/ μ g and the RF/IgA ratio

TABLE 2 Periodontal characteristics

Periodontal characteristics	RA (n = 72)	HC (n = 151)	p value
Number of teeth (median, IQR)	26 (22–28)	28 (25–30)	.0003
PISA in mm ² (median, IQR)	4.9 (0–98)	11 (0–186)	.42
Periodontal disease (%) (n) according to cut-off of PISA ≥ 130 mm ^{2a}	21 (15)	29 (44)	.1992
% of sites with pocket probing depth 1–2 mm (median, IQR)			
All	95 (74–99)	92 (66–100)	
With periodontal disease	56 (42–71)	55 (35–68)	
Without periodontal disease	97 (94–99)	98 (91–100)	
% of sites with pocket probing depth 3–4 mm (median, IQR)			
All	4.7 (0.8–24)	6.2 (0–27)	
With periodontal disease	41 (27–44)	36 (21–45)	
Without periodontal disease	2.3 (0.6–6.1)	1.7 (0–7.9)	
% of sites with pocket probing depth ≥ 5 mm (median, IQR)			
All	0 (0–0.5)	0 (0–3.8)	
With periodontal disease	3.8 (1.1–13)	6.5 (2.8–15)	
Without periodontal disease	0 (0–0)	0 (0–0)	
% of sites with bleeding on probing (median, IQR)			
All	1.1 (0–9.8)	1.3 (0–13)	
With periodontal disease	35 (18–57)	31 (15–45)	
Without periodontal disease	0.6 (0–2.0)	0 (0–2.5)	

^aA PISA value ≥ 130 mm² has been shown to be associated with the CDC-AAP case definition of periodontitis (Leira et al., 2018).

was 0.691 U/μg, while in the HC they were ACPA/IgA: 12.378 U/μg and RF/IgA: 0.541 U/μg. Thus, when relating the autoantibody levels to total IgA, the IgA-ACPA in the GCF of the HC seems to be over represented compared with IgA RF. When looking at the individuals with high (>0.1 U/ml) IgA-ACPA (n = 27) or low (<0.1 U/ml) IgA-ACPA (n = 124), it was found that there were several differences. Although the PISA difference was borderline between these groups (p = .058), the total levels of IgG and IgA in the GCF were higher in the IgA-ACPA high group (p = .0066 and 0.0084, respectively).

The relationship between the PISA and IgA-ACPA in the GCF may be also reflected by the higher prevalence of IgA-ACPA (>0.1 U/ml) in the HC with periodontal disease (25%) than without periodontal disease (15%; Figure 1c). The HC smokers tended to have higher levels of IgA-ACPA in the GCF than the non-smokers (26% vs. 15%).

The ACPA and RF levels in the RA patients' serum and GCF were highly correlated (p < .0001 for all antibodies), in contrast to the HC (only slightly significant for IgA-ACPA, p = .050). All the participants' total IgG and total IgA were highly inter-correlated (p < .0001). The total IgG, total IgA, and IL-8 in the GCF were most strongly correlated with the PISA of the HC (p < .0001 for total IgG and total IgA, p = .0042 for IL-8), which is also reflected in Figure 2.

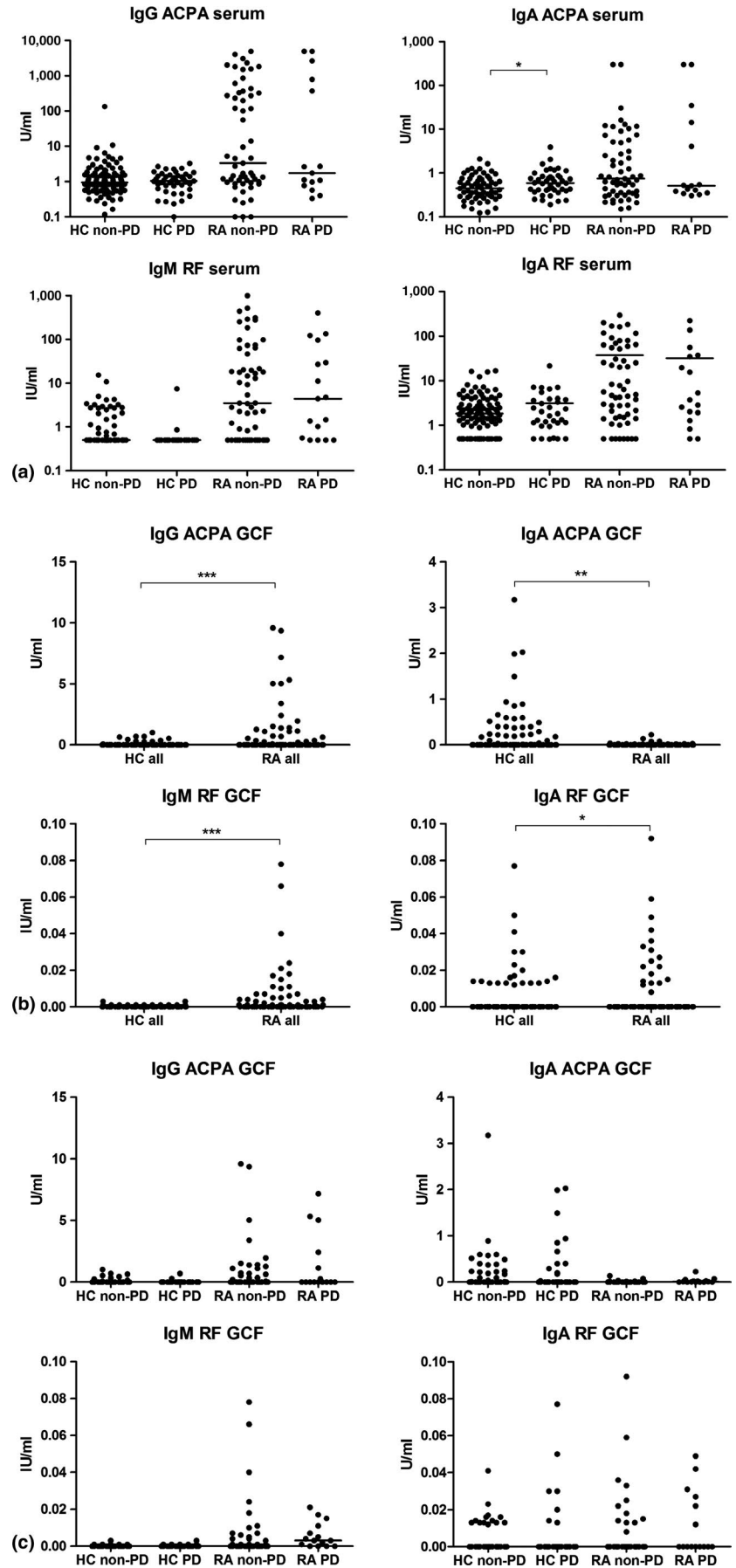
4 | DISCUSSION

This study is the first to assess the IgA isotypes of ACPA and RF in the periodontal exudate (GCF) from a large cohort of Indonesian

RA patients and Indonesian individuals without RA (HC). The prevalence of periodontal inflammation was equal between these groups. As expected, the seropositivity of ACPA and RF was higher in the RA patients. Regarding GCF, however, the IgA isotype of the ACPA was higher in the HC, whereas the IgG isotype of the ACPA was higher in the GCF of the RA patients. Since the ACPA and RF levels in the RA patients' GCF were highly correlated to the levels in the serum, the presence of these autoantibodies in the GCF may partially be derived from the circulation. There were no correlations found between autoantibodies in serum and GCF in the HC. These observations indicate that IgA-ACPA may have been induced locally in the HCs' mucosal periodontal tissues.

We showed previously that the levels of RA autoantibodies (ACPA and RF) of the IgA isotype in the serum of non-RA patients with mucosal inflammation, such as bronchiectasis, cystic fibrosis, and periodontal disease, were higher compared to those of healthy people (Janssen et al., 2015). Several studies have investigated the use of measuring the isotypes of the RA autoantibodies in RA risk and RA patients. In general, testing for multiple ACPA specificities and more isotypes increases the possibility of predicting RA and increases the diagnostic power of autoimmune serology (Brink et al., 2016; Sieghart et al., 2018). Ärlestig et al. (2012) found that first-degree relatives of RA patients have a higher relative distribution of IgA and IgM ACPA isotypes than IgG, whereas the IgG isotype dominates in patients with RA. Another study looked at ACPA isotypes and secretory IgA (S-IgA) ACPA levels in the serum of recent-onset RA patients, with a 3-year follow-up (Kastbom

FIGURE 1 ACPA and RF in the serum (a) and GCF (b) of RA patients and healthy controls (HC) with or without periodontal disease (PD) (c). Lines represent medians. * $p < .05$, ** $p < .01$, *** $p < .0001$



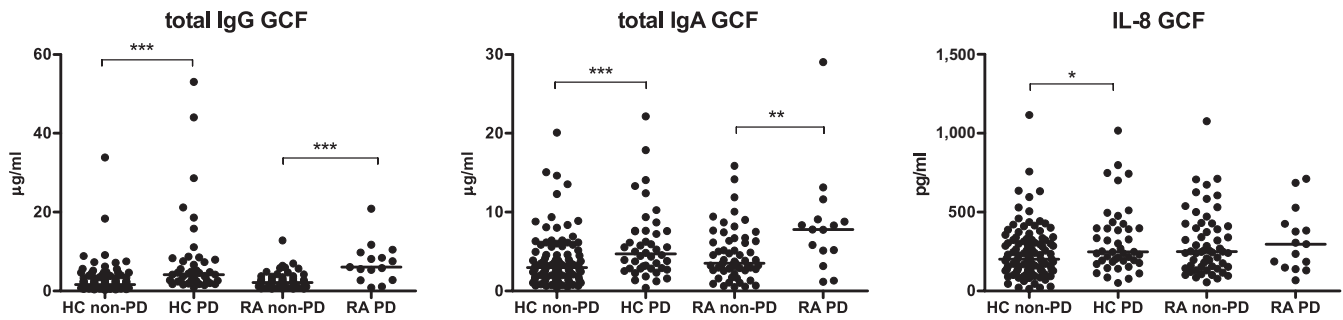


FIGURE 2 Total IgG, total IgA, and IL-8 in GCF of RA patients and healthy controls (HC) with or without periodontal disease (PD). * $p < .05$, ** $p < .01$, *** $p < .001$. Lines represent medians

et al., 2018). It was reported that the S-IgA and IgM ACPA levels declined rapidly with anti-rheumatic therapy and correlated with decreased disease activity, indicating that the down-regulation of mucosal immunity to citrullinated proteins or peptides may be a key feature of therapy response in early RA. Finally, salivary IgA anti-cyclic citrullinated protein (CCP) was measured in unstimulated salivary samples from 63 patients with established RA and from 20 healthy people (Svärd, Kastbom, Sommarin, & Skogh, 2013). Salivary IgA anti-CCP was found in 14/63 (22%) patients and one (5%) control, and was associated with a less severe outcome of RA. Hence, relatively higher levels of IgA-ACPA are seen in pre- and recent-onset RA patients than IgG ACPA, and may be predictive of RA development and severity. Of note, Aleyd, Al, Tuk, van der Laken, and van Egmond (2016) demonstrated that IgA complexes in the plasma and synovial fluid of patients with RA induce neutrophil extracellular traps via Fc α RI, indicating that IgA autoantibodies may play a role in hypercitrullination, induced by NETs.

Recently Holers et al. (2018) published an interesting review discussing the mucosal origin theory: protection turns to destruction. In their conclusion, they state that IgA-ACPA plays an important role in the local protective immune response, outside its eventual causal relationship with inflammatory arthritis. Many potential factors in the mucosa, as well as systemic alterations, could influence both the local initiation and systemic expansion of ACPA expression. Holers et al. (2018) stated that a likely major driver of early disease initiation is the loss of the mucosal barrier which, here, concerns the oral mucosa in relation to periodontal disease.

The extent of periodontal inflammation in our study was quantified by the PISA. The high correlation between total IgG and total IgA in the GCF with the PISA in all the participants underlines that the PISA is a good clinical measure for quantifying the burden of periodontal inflammation. As radiographic assessment is necessary to confirm periodontal diagnosis, PISA does not reflect periodontal diagnosis. Therefore, the division in presence or absence of periodontitis according to the cut-off values is somewhat artificial. In addition, prevalence of periodontitis according to PISA values was relatively low in our study groups.

The correlation of IL-8 with PISA in the HC supports the importance of neutrophil involvement in periodontal inflammation (Ling

et al., 2015). The presence of RA, or RA medication, may have overshadowed this relationship in the RA patients. In addition, the relationships between IgA-ACPA in the GCF, PISA, and IL-8 in the HC may point towards the involvement of hypercitrullination and NET formation in these neutrophils (Cooper et al., 2013). As mentioned previously, IL-8 may play a role in attracting neutrophils and later in inducing NET formation (Gonzalez-Aparicio & Alfaro, 2019). Another factor influencing citrullination and ACPA induction is smoking (Sakkas, Bogdanos, Katsiari, & Platsoucas, 2014). In our study, the influence of smoking was reflected by the high IgA-ACPA in the GCF of the HC smokers compared with the HC who had never smoked.

Although disease activity was high in the RA patients of our study population, their seropositivity for ACPA and RF was rather low, which can be regarded as a limitation of this study. ACPA and RF seropositivity has been reported to be comparable between Asian and Western populations (around 60%–80%; Barra et al., 2014; Chun-Lai et al., 2011; Terao et al., 2015). Our population was of Indonesian decent and many more men than women smoke, which may explain that fewer smokers were present in the RA group, and also that the prevalence of periodontal disease was not different between the RA patients and the HC. The high RA disease activity can be explained by the fact that biological treatment is not accessible for many patients in this country due to high costs (Fia & Westra, 2018). Another limitation might be that the collecting and processing techniques of GCF samples are sensitive, which should be taken into account (Lamster & Ahlo, 2007). Finally, we did not investigate the relationships between specific compositions of the subgingival microbial biofilm in this study but this will be investigated in our future studies.

Within in the limitations of this study, the presence of IgA-ACPA and the absence of IgG ACPA in the GCF of HC point towards local induction of these autoantibodies. This local induction is probably, amongst others, related to chronic mucosal inflammation due to periodontal disease or smoking.

ACKNOWLEDGEMENTS

The authors thank Kuniyasari Lisa MD and Dwi Budi Darmawati MD for the clinical examinations of the RA patients, and Miftah Darmawanti DMD and Amelia Elisabeth Pranoto DMD for the oral examinations of all the participants.

CONFLICT OF INTEREST

All the authors have nothing to disclose.

ORCID

Menke de Smit  <https://orcid.org/0000-0003-1863-0775>

Arjan Vissink  <https://orcid.org/0000-0003-2581-4361>

Johanna Westra  <https://orcid.org/0000-0002-6581-6508>

REFERENCES

- Aletaha, D., Neogi, T., Silman, A. J., Funovits, J., Felson, D. T., Bingham, C. O., ... Hawker, G. (2010). 2010 Rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis and Rheumatism*, 62(9), 2569–2581. <https://doi.org/10.1002/art.27584>
- Aleyd, E., Al, M., Tuk, C. W., van der Laken, C. J., & van Egmond, M. (2016). IgA complexes in plasma and synovial fluid of patients with rheumatoid arthritis induce neutrophil extracellular traps via FcαRI. *The Journal of Immunology*, 197(12), 4552–4559. <https://doi.org/10.4049/jimmunol.1502353>
- Ärlestig, L., Mullazehi, M., Kokkonen, H., Rocklöv, J., Rönnelid, J., & Dahlqvist, S. R. (2012). Antibodies against cyclic citrullinated peptides of IgG, IgA and IgM isotype and rheumatoid factor of IgM and IgA isotype are increased in unaffected members of multicase rheumatoid arthritis families from northern Sweden. *Annals of the Rheumatic Diseases*, 71(6), 825–829. <https://doi.org/10.1136/annrheumdis-2011-200668>
- Barra, L., Pope, J. E., Orav, J. E., Boire, G., Haraoui, B., Hitchon, C., ... Bykerk, V. P. (2014). Prognosis of seronegative patients in a large prospective cohort of patients with early inflammatory arthritis. *The Journal of Rheumatology*, 41(12), 2361–2369. <https://doi.org/10.3899/jrheum.140082>
- Brink, M., Hansson, M., Mathsson-Alm, L., Wijayatunga, P., Verheul, M. K., Trouw, L. A., ... Rantapää-Dahlqvist, S. (2016). Rheumatoid factor isotypes in relation to antibodies against citrullinated peptides and carbamylated proteins before the onset of rheumatoid arthritis. *Arthritis Research & Therapy*, 18(1), 43. <https://doi.org/10.1186/s13075-016-0940-2>
- Champagne, C. M. E., Buchanan, W., Reddy, M. S., Preisser, J. S., Beck, J. D., & Offenbacher, S. (2003). Potential for gingival crevice fluid measures as predictors of risk for periodontal diseases. *Periodontology* 2000, 31, 167–180. <https://doi.org/10.1034/j.1600-0757.2003.03110.x>
- Chun-Lai, T., Padyukov, L., Dhaliwal, J. S., Lundström, E., Yahya, A., Muhamad, N. A., ... Malaysian Epidemiological Investigation of Rheumatoid Arthritis (MyEIRA) Study Group (2011). Shared epitope alleles remain a risk factor for anti-citrullinated proteins antibody (ACPA) – Positive rheumatoid arthritis in three Asian ethnic groups. *PLoS ONE*, 6(6), e21069. <https://doi.org/10.1371/journal.pone.0021069>
- Cooper, P. R., Palmer, L. J., & Chapple, I. L. C. (2013). Neutrophil extracellular traps as a new paradigm in innate immunity: Friend or foe? *Periodontology* 2000, 63(1), 165–197. <https://doi.org/10.1111/prd.12025>
- de Pablo, P., Dietrich, T., Chapple, I. L. C., Milward, M., Chowdhury, M., Charles, P. J., ... Venables, P. J. (2014). The autoantibody repertoire in periodontitis: A role in the induction of autoimmunity to citrullinated proteins in rheumatoid arthritis? *Annals of the Rheumatic Diseases*, 73(3), 580–586. <https://doi.org/10.1136/annrheumdis-2012-202701>
- Fia, F., & Westra, J. (2018). Overview of rheumatoid arthritis therapy: Management options in Indonesia. *Tarumanagara Medical Journal*, 1(1), 208–220.
- Gargiulo, A. V., Robinson, J., Toto, P. D., & Gargiulo, A. W. (1982). Identification of rheumatoid factor in periodontal disease. *Journal of Periodontology*, 53(9), 568–577. <https://doi.org/10.1902/jop.1982.53.9.568>
- Gonzalez-Aparicio, M., & Alfaro, C. (2019). Influence of interleukin-8 and neutrophil extracellular trap (NET) formation in the tumor microenvironment: Is there a pathogenic role? *Journal of Immunology Research*, 2019, 1–7. <https://doi.org/10.1155/2019/6252138>
- Harvey, G. P., Fitzsimmons, T. R., Dhamarpatni, A. A. S. K., Marchant, C., Haynes, D. R., & Bartold, P. M. (2013). Expression of peptidylarginine deiminase-2 and -4, citrullinated proteins and anti-citrullinated protein antibodies in human gingiva. *Journal of Periodontal Research*, 48(2), 252–261. <https://doi.org/10.1111/jre.12002>
- Hendler, A., Mulli, T. K., Hughes, F. J., Perrett, D., Bombardieri, M., Hourihaddad, Y., ... Nissim, A. (2010). Involvement of autoimmunity in the pathogenesis of aggressive periodontitis. *Journal of Dental Research*, 89(12), 1389–1394. <https://doi.org/10.1177/0022034510381903>
- Hirsch, H. Z., Tarkowski, A., Koopman, W. J., & Mestecky, J. (1989). Local production of IgA- and IgM-rheumatoid factors in adult periodontal disease. *Journal of Clinical Immunology*, 9(4), 273–278. <https://doi.org/10.1007/BF00918658>
- Holers, V. M., Demouelle, M. K., Kuhn, K. A., Buckner, J. H., Robinson, W. H., Okamoto, Y., ... Deane, K. D. (2018). Rheumatoid arthritis and the mucosal origins hypothesis: Protection turns to destruction. *Nature Reviews Rheumatology*, 14(9), 542–557. <https://doi.org/10.1038/s41584-018-0070-0>
- Janssen, K. M. J., de Smit, M. J., Brouwer, E., de Kok, F. A. C., Kraan, J., Altenburg, J., ... Westra, J. (2015). Rheumatoid arthritis-associated autoantibodies in non-rheumatoid arthritis patients with mucosal inflammation: A case-control study. *Arthritis Research & Therapy*, 17, 174. <https://doi.org/10.1186/s13075-015-0690-6>
- Kastbom, A., Roos Ljungberg, K., Ziegelasch, M., Wetterö, J., Skogh, T., & Martinsson, K. (2018). Changes in anti-citrullinated protein antibody isotype levels in relation to disease activity and response to treatment in early rheumatoid arthritis. *Clinical & Experimental Immunology*, 194(3), 391–399. <https://doi.org/10.1111/cei.13206>
- Konig, M. F., Abusleme, L., Reinholdt, J., Palmer, R. J., Teles, R. P., Sampson, K., ... Andrade, F. (2016). Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. *Science Translational Medicine*, 8(369), 369ra176. <https://doi.org/10.1126/scitranslmed.aaj1921>
- Kumar, P. S. (2016). From focal sepsis to periodontal medicine: A century of exploring the role of the oral microbiome in systemic disease. *The Journal of Physiology*, 595, 465–476. <https://doi.org/10.1113/JP272427>
- Lamster, I. B., & Ahlo, J. K. (2007). Analysis of gingival crevicular fluid as applied to the diagnosis of oral and systemic diseases. *Annals of the New York Academy of Sciences*, 1098(1), 216–229. <https://doi.org/10.1196/annals.1384.027>
- Laugisch, O., Wong, A., Sroka, A., Kantyka, T., Koziel, J., Neuhaus, K., ... Eick, S. (2016). Citrullination in the periodontium—A possible link between periodontitis and rheumatoid arthritis. *Clinical Oral Investigations*, 20(4), 675–683. <https://doi.org/10.1007/s00784-015-1556-7>
- Leira, Y., Martín-Lancharro, P., & Blanco, J. (2018). Periodontal inflamed surface area and periodontal case definition classification. *Acta Odontologica Scandinavica*, 76(3), 195–198. <https://doi.org/10.1080/00016357.2017.1401659>
- Li, P., Li, M., Lindberg, M. R., Kennett, M. J., Xiong, N., & Wang, Y. (2010). PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. *The Journal of Experimental Medicine*, 207(9), 1853–1862. <https://doi.org/10.1084/jem.20100239>
- Ling, M. R., Chapple, I. L., & Matthews, J. B. (2015). Peripheral blood neutrophil cytokine hyper-reactivity in chronic periodontitis. *Innate Immunity*, 21(7), 714–725. <https://doi.org/10.1177/1753425915589387>
- Malmström, V., Catrina, A. I., & Klareskog, L. (2017). The immunopathogenesis of seropositive rheumatoid arthritis: From triggering

- to targeting. *Nature Reviews Immunology*, 17(1), 60–75. <https://doi.org/10.1038/nri.2016.124>
- Mikuls, T. R., Payne, J. B., Deane, K. D., & Thiele, G. M. (2016). Autoimmunity of the lung and oral mucosa in a multisystem inflammatory disease: The spark that lights the fire in rheumatoid arthritis? *The Journal of Allergy and Clinical Immunology*, 137(1), 28–34. <https://doi.org/10.1016/j.jaci.2015.10.024>
- Nesse, W., Abbas, F., van der Ploeg, I., Spijkervet, F. K. L., Dijkstra, P. U., & Vissink, A. (2008). Periodontal inflamed surface area: Quantifying inflammatory burden. *Journal of Clinical Periodontology*, 35(8), 668–673. <https://doi.org/10.1111/j.1600-051X.2008.01249.x>
- Nesse, W., Westra, J., van der Wal, J. E., Abbas, F., Nicholas, A. P., Vissink, A., & Brouwer, E. (2012). The periodontium of periodontitis patients contains citrullinated proteins which may play a role in ACPA (anti-citrullinated protein antibody) formation. *Journal of Clinical Periodontology*, 39(7), 599–607. <https://doi.org/10.1111/j.1600-051X.2012.01885.x>
- Nociti, F. H., Casati, M. Z., & Duarte, P. M. (2015). Current perspective of the impact of smoking on the progression and treatment of periodontitis. *Periodontology 2000*, 67(1), 187–210. <https://doi.org/10.1111/prd.12063>
- Potempa, J., Mydel, P., & Koziel, J. (2017). The case for periodontitis in the pathogenesis of rheumatoid arthritis. *Nature Reviews Rheumatology*, 13(10), 606–620. <https://doi.org/10.1038/nrrheum.2017.132>
- Prevo, M. L., van't Hof, M. A., Kuper, H. H., van Leeuwen, M. A., van de Putte, L. B., & van Riel, P. L. (1995). Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis and Rheumatism*, 38(1), 44–48.
- Rahajoe, P. S., de Smit, M. J., Kertia, N., Westra, J., & Vissink, A. (2019). Cytokines in gingivocrevicular fluid of rheumatoid arthritis patients: A review of the literature. *Oral Diseases*, 25(6), 1423–1434. <https://doi.org/10.1111/odi.13145>
- Rosenstein, E. D., Greenwald, R. A., Kushner, L. J., & Weissmann, G. (2004). Hypothesis: The humoral immune response to oral bacteria provides a stimulus for the development of rheumatoid arthritis. *Inflammation*, 28(6), 311–318. <https://doi.org/10.1007/s10753-004-6641-z>
- Sakkas, L. I., Bogdanos, D. P., Katsiari, C., & Platsoucas, C. D. (2014). Anti-citrullinated peptides as autoantigens in rheumatoid arthritis—Relevance to treatment. *Autoimmunity Reviews*, 13(11), 1114–1120. <https://doi.org/10.1016/j.autrev.2014.08.012>
- Schwenzer, A., Quirke, A.-M., Marzeda, A. M., Wong, A., Montgomery, A. B., Sayles, H. R., ... Midwood, K. S. (2017). Association of distinct fine specificities of anti-citrullinated peptide antibodies with elevated immune responses to *Prevotella intermedia* in a subgroup of patients with rheumatoid arthritis and periodontitis. *Arthritis & Rheumatology*, 69(12), 2303–2313. <https://doi.org/10.1002/art.40227>
- Sieghart, D., Platzer, A., Studenic, P., Alasti, F., Grundhuber, M., Swiniarski, S., ... Steiner, G. (2018). Determination of autoantibody isotypes increases the sensitivity of serodiagnostics in rheumatoid arthritis. *Frontiers in Immunology*, 9, 876. <https://doi.org/10.3389/fimmu.2018.00876>
- de Smit, M., Westra, J., Vissink, A., Doornbos-van der Meer, B., Brouwer, E., van Winkelhoff, A. J., ... van Winkelhoff, A. J. (2012). Periodontitis in established rheumatoid arthritis patients: A cross-sectional clinical, microbiological and serological study. *Arthritis Research & Therapy*, 14(5), R222. <https://doi.org/10.1186/ar4061>
- Svärd, A., Kastbom, A., Sommarin, Y., & Skogh, T. (2013). Salivary IgA antibodies to cyclic citrullinated peptides (CCP) in rheumatoid arthritis. *Immunobiology*, 218(2), 232–237. <https://doi.org/10.1016/j.imbio.2012.04.011>
- Terao, C., Yamakawa, N., Yano, K., Markusse, I. M., Ikari, K., Yoshida, S., ... Matsuda, F. (2015). Rheumatoid factor is associated with the distribution of hand joint destruction in rheumatoid arthritis. *Arthritis & Rheumatology*, 67(12), 3113–3123. <https://doi.org/10.1002/ART.39306>
- Thé, J., & Ebersole, J. L. (1991). Rheumatoid factor (RF) distribution in periodontal disease. *Journal of Clinical Immunology*, 11(3), 132–142.
- van der Helm-van Mil, A. H., Verpoort, K. N., Breedveld, F. C., Toes, R. E., & Huizinga, T. W. (2005). Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Research & Therapy*, 7(5), R949. <https://doi.org/10.1186/ar1767>
- Van Leeuwen, M. A., Westra, J., van Riel, P. L. C. M., Limburg, P. C., & Van Rijswijk, M. H. (1995). IgM, IgA, and IgG rheumatoid factors in early rheumatoid arthritis predictive of radiological progression? *Scandinavian Journal of Rheumatology*, 24(3), 146–153. <https://doi.org/10.3109/03009749509099303>
- Wegner, N., Wait, R., Sroka, A., Eick, S., Nguyen, K.-A., Lundberg, K., ... Venables, P. J. (2010). Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and α -enolase: Implications for autoimmunity in rheumatoid arthritis. *Arthritis & Rheumatism*, 62(9), 2662–2672. <https://doi.org/10.1002/art.27552>
- Wells, G., Becker, J., Teng, J., Dougados, M., Schiff, M., Smolen, J., ... van Riel, P. (2009). Validation of the 28-joint disease activity score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythr. *Annals of the Rheumatic Diseases*, 68(6), 954–960.

How to cite this article: Rahajoe PS, de Smit M, Schuurmans G, et al. Increased IgA anti-citrullinated protein antibodies in the periodontal inflammatory exudate of healthy individuals compared to rheumatoid arthritis patients. *J Clin Periodontol*. 2020;47:552–560. <https://doi.org/10.1111/jcpe.13277>