Periodontitis prevalence and serum antibody reactivity to periodontal bacteria in primary Sjögren's syndrome

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Periodontitis prevalence and serum antibody reactivity to periodontal bacteria in primary

Sjögren's syndrome: a pilot study

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Abstract

Aims: 1. to assess the prevalence of periodontitis among patients with primary Sjögren's syndrome
(pSS) and comparator groups of patients with rheumatoid arthritis (RA) and osteoarthritis (OA). 2. To
perform a pilot study to compare serum antibody responses to 10 oral/periodontal bacteria in these
patient groups and a historical comparator group of patients with periodontitis.

Materials and Methods: Standard clinical periodontal assessments were performed on 39 pSS, 36

RA and 23 OA patients and "In-house" antibody ELISAs for serum antibodies against 10

oral/periodontal bacteria were performed in these groups.

Results: 46% of the pSS group, 64% of the RA group and 48% of the OA group had moderate/severe

periodontitis. These frequencies did not reach statistical significance between groups. Raised

antibody levels to *P. denticola* were found in the pSS, RA and periodontitis groups compared to the

OA group. Significant between group differences were seen for *A. actinomycetemcomitans, P.*

intermedia, and *C. showae.* None of these differences were specifically associated with pSS.

Conclusion: This study showed no increase in periodontitis in pSS patients. Although the P. denticola

data is of interest, identifying bacterial triggering factors for pSS will likely require alternative

strategies including modern techniques such as microbiome analysis.

Clinical Relevance

Scientific rationale for the study: The triggering factors for Sjögren's syndrome are unknown. The

close association with anti-Ro/La antibodies could indicate an antigen-driven process. Our

hypotheses are that 1. Oral or periodontal bacteria could be a driver of antigen-specific responses

leading to cross-reactivity with autoantigens such as Ro and La and 2. An increased prevalence of

periodontitis could support a permissive pro-inflammatory environment for autoantigenicity leading

to Sjögren's syndrome.

Principal findings: There was no increased prevalence of periodontitis in Sjögren's syndrome in this

study. Raised anti-bacterial antibody levels were seen but were not disease-specific.

Practical implications: Alternative molecular microbial analyses may offer a more effective route to

address these key questions

INTRODUCTION

Primary Sjögren's syndrome (pSS) is an autoimmune disease characterized by inflammation of the secretory glands leading to reduced/absent saliva and tear production (Jonsson et al 2005). PSS typically affects women in their middle years. Oral features of dryness (xerostomia) and of salivary gland swelling are prominent features of pSS and the parotid salivary glands are most likely to be enlarged and most likely to develop B-cell mucosa-associated lymphoid tissue (MALT) lymphoma (Jonsson et al 2005).

70% of pSS patients have autoantibodies against the Ro and/or La antigens and 90% of the patients with high levels of both of these antibodies are HLA-DR3, DQ2 positive (Davies et al 2002). These patients are at particular risk of developing other complications (systemic pSS) such as salivary gland swelling, peripheral neuropathy, interstitial lung disease, arthritis, skin vasculitis and lymphoma (Jonsson et al 2005).

On histological examination, the salivary glands in pSS are characterized by collections (foci) of lymphocytes located around the salivary ducts through which saliva passes into the mouth suggesting that the ducts play an important role in the process that drives the local immune response underpinning the glandular features of the condition.

Since HLA-DR molecules classically present antigens to helper CD4+ T-lymphocytes, one logical hypothesis that brings together the above data is that a limited set of HLA-DR3,DQ2 restricted antigens, derived from, or presented by, ductal epithelial cells, generate an inflammatory T-helper (CD4+) lymphocyte response, the consequence of which is inhibition of gland secretion and, over time, the clinical picture of pSS. The CD4 positive T cells also generate a T cell dependent antibody response resulting in the production of anti-Ro and anti-La autoantibodies. There is considerable evidence for B- T cell interaction and development of tertiary lymphoid tissue in pSS. (Amft et al 2001, Barone et al 2008).

The antigenic stimulus, however, is unknown. Most attention has been paid to viruses such as Epstein-Barr virus, human T-cell leukaemia virus 1 and hepatitis C virus (Delaleu et al 2008), but the strength of evidence for a viral aetiology is modest. An alternative view, as hypothesized here, is that oral bacteria, such as those originating from the periodontal biofilm might trigger the disease in susceptible individuals with a permissive genetic background (HLA-DR3 DQ2) through cross-reactive immune responses to self-antigens. In support of this hypothesis that bacterial antigen presentation by HLA class II molecules can drive human inflammatory diseases, *H. pylori* has been identified as a bacterial cause of peptic ulcer disease (Marshall and Warren 1984), which is also associated with MALT lymphoma. Although the antigens are not of bacterial origin, coeliac disease is now known to be triggered by HLA DQ2/8 restricted peptides derived from gluten typically of wheat origin (Dieterich et al 1997). Rheumatoid arthritis (RA) is strongly associated with HLA-DR4 and to a lesser extent with HLA-DR1 ('shared epitope hypothesis') (Gregerson et al 1987). This association has been known for many decades, but, it is only recently that a likely explanation has been proposed through

the presentation of citrullinated peptides (CCP) (Schellekens et al 2000) by HL-DR molecules (Van Gaalen et al 2004). Furthermore, periodontal bacteria may play a key role in the initiation of rheumatoid arthritis through citrullination as a mechanism of generating antigenic peptides (de Pablo et al 2009). The periodontal bacterial species most strongly associated with RA are *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia and Aggregatibacter actinomycetemcomitans* (Mikuls et al 2008). What is of particular interest is that *P. gingivalis* produces an enzyme, homologous to human peptidyl arginine deaminase (PAD), called PPAD, which citrullinates peptide antigens. Individuals with periodontitis are exposed, therefore, to citrullinated antigens that may become systemic immunogens in susceptible individuals (Lundberg et al 2008). The levels of antibodies against *P. gingivalis* correlate with anti-CCP antibody levels in patients with RA in at least some studies (Mikuls et al 2008). Antibodies to citrullinated α-enolase are also specific for RA and an immuno-dominant epitope in this protein shows sequence similarity and cross-reactivity with *P. gingivalis* enolase (Lundberg et al 2008). 10% of patients with RA also develop a Sjögren's-like syndrome ('secondary SS').

If periodontal or other oral bacteria have a pathogenic role in RA, then it is logical to propose that similar processes could occur in pSS where the focus of inflammation is the salivary ducts, which is the point of contact between the salivary glands and the mouth. Our principle hypothesis is that raised antibody levels in pSS patients' serum could identify bacteria potentially involved in triggering the disease. In order to address this we have investigated the prevalence of antibody reactivity to whole bacterial lysates of 10 selected oral/periodontal bacteria in pSS patients compared to comparator groups of patients with osteoarthritis (OA) without inflammatory arthritis, or RA patients attending the same clinics and serum from a historical group of periodontitis patients.

Periodontitis is the most common chronic inflammatory disease of humans (McGraw et al 1999), characterised by a dysregulation of the immune-inflammatory response to periodontal bacterial pathogens within the sub-gingival biofilm. Our second more general hypothesis is that

higher levels of periodontitis in pSS patients contribute to a pro-inflammatory environment that could in turn facilitate antigen-specific immune responses. In this study, in order to address this hypothesis, we have evaluated the prevalence of periodontitis in the above pSS, RA and OA groups.

METHODS

Patients

The study was carried out following Research Ethics Committee approval and all patients gave informed written consent. Convenience samples of patients with pSS, RA and OA attending the Rheumatology clinics at the Queen Elizabeth Hospital Birmingham were recruited during 2009-2013. Patients with periodontitis who had previously been recruited to a clinical trial at the University of Birmingham School of Dentistry (Chapple et al 2012)) were asked if their serum could be used for this study. All suitable patients with pSS, RA and OA were invited to participate in both the bacterial antibody reactivity component of the study and the periodontal assessment. Edentulous patients were excluded from the periodontal assessment.

Periodontal Assessment

Patients who agreed to do so underwent a detailed periodontal assessment by a calibrated research hygienist (DS) at Birmingham Dental Hospital under the direction of a consultant clinician (ILCC). Mean probing depth, mean attachment loss and cumulative probing depth (the sum of the deepest pathological pocket depths (PPD 4+ mm) on each tooth present) were calculated. Patients were assigned a diagnosis of periodontitis based on the American Academy of Periodontology/Centers for Disease Control (AAP/CDC) definition of

periodontitis (0 - none/mild, 1 - moderate, 2 - severe), whereby moderate disease is defined as at least 2 teeth with interproximal clinical attachment loss (CAL) of 4+mm or at least 2 teeth with interproximal PPD 5+mm, and severe is defined as at least 2 teeth with interproximal CAL 6+mm and at least one tooth with interproximal PPD 5+mm (Page and Eke 2007). This is a widely practised approach (Dietrich et al 2008).

Bacteria studied

A pragmatic decision was made to evaluate 10 strains of oral bacteria from the American type culture collection (ATCC, Rockville USA) (Roberts et al 2002) in this pilot study. Six of these are reported to be associated with periodontitis: *Porphyromonas gingivalis* (ATCC 33277); *Prevotella denticola* (ATCC 35308*); Aggregatibacter actinomycetemcomitans (*ATCC 43718); *Fusobacterium nucleatum* (ATCC 10953*); Campylobacter showae (*ATCC 51146) and *Prevotella intermedia* (ATCC 25611). Four other oral bacteria were also evaluated as a comparison: *Capnocytophaga gingivalis (*ATCC 33624 – normal oral flora found in supra and subgingival plaque); *Enterococcus faecalis (*ATCC 29212 – commensal gastrointestinal tract bacteria that can also be found in the oral cavity, associated with endodontic lesions and in the sub gingival plaque & saliva); *Streptococcus sanguis* (ATCC 10556 – normal oral flora) and *Streptococcus mutans* (ATCC 25175 – associated with dental caries). These bacteria were chosen to re-assess previous data of interest (e.g. *P. gingivalis*, *A. actinomycetemcomitans, P. intermedia)* and to provide a spread of relevant periodontal and non-periodontal species based on Socransky's complexes (Socransky et al 2002).

Bacterial sample preparation

Initially bacteria were inoculated into broth media (type dependent on the organism; Brain heart infusion broth with 10% serum for fastidious bacteria, or typtone soya broth, Oxoid, UK). Suspensions were incubated at $37^{o}C$ anaerobically or in 5% CO_2 until turbid (24-72hrs), then sub-

cultured onto agar plates (Fastidious anaerobe agar or 10% horse blood agar number 2; Oxoid, UK) and re-incubated using identical conditions. Agar plates were checked for typical colonial morphology and Gram stained to ensure purity. Morphologically identical colonies were then sub-cultured into liquid media (Brain heart infusion broth with 10% serum for fastidious bacteria, or typtone soya broth. Oxoid, UK) and incubated at $37^{o}C$ anaerobically or in 5% CO_2 until turbid (24-72h). The resulting broth cultures were centrifuged at 3,000 RPM for 10 mins, supernatant removed and the bacterial pellet washed 3 times with 20 ml Phosphate buffered saline (PBS) (Invitrogen, UK) before finally re-suspending in 20 ml PBS. The optical density (OD) was determined at 600 nm using a Jenway spectrophotometer (Dunmow, UK). The resulting suspension was heat treated ($100^{o}C$ for 10 mins) with re-plating experiments performed to ensure effective bacterial killing. Suspensions were stored at $-20^{o}C$ prior to use. Bacteria were sonicated prior to use using a Sonics VibraCell Ultrasonic Processor. The protein concentration of sonicated lysates was determined using Pierce ThermoScientific BCA Assay Kit. All assays were performed as per the manufacturer's instructions. Absorbance was read at 562 nm; protein concentrations were calculated using the in-kit standard curve.

ELISAs

The ELISA protocol was optimised for each individual strain of bacteria. The bacterial lysate and patient sera were titrated to determine the optimal dilution for the ELISA procedure. Each bacterial lysate was titrated from 5 µg/ml to 0.625 µg/ml; patient sera were titrated in doubling dilutions from 1:100 to 1:25600.

The ELISA protocol was adapted from previously published work (Mikuls et al 2008, Engvall and Perlman 1971). Briefly, sonicated bacterial lysates were prepared at the required concentration with Carbonate Bicarbonate Buffer (0.05M; pH 9.6; Sigma Aldrich), then filtered through a CellTrac

50 µm filter to remove any bacterial clumps. F96 Nunc Microwell Plates were coated with 100 µl of the bacterial lysate at the required concentration (1-2.5 µg/ml) and left overnight at 4°C in a humid chamber. The plates were washed 2x with 200 µl Phosphate Buffered Saline Tween (PBST, 0.05%, pH 7.4) and were then blocked for 30 minutes with 200 µl PBS/1% Bovine Serum Albumin (BSA) at 37°C; and washed 4x with 200 µl PBST.

100 µl Patient Sera serially diluted 1:100, 1:1000 and 1:10000 were added and plates were incubated for 1 hour at 37°C in a humid chamber. Plates were washed 4x with PBST, and incubated at 37°C with 100 µl of Peroxidase Labelled Goat Anti-Human IgG (Sigma Aldrich) at a concentration of 1:1000 (diluted in PBS/1% BSA). Plates were washed 4x with PBST, after which 100 µl of o-Phenylenediamine dihydrochloride (OPD) substrate (Dako, prepared according to manufacturer's instructions) was added to each well and incubated for 30 minutes in the dark at room temperature. The reaction was stopped with 100 µl 2M sulphuric acid, and the absorbance of each well was measured at 492 nm using an Anthos HTIII plate reader. A Pooled Reference Serum at concentrations of 1:100, 1:1000 and 1:10,000 was included on each plate, along with blank wells with no primary sera. All samples were normalized to the Standard Reference Serum on the same plate to ensure consistency of results. Concentrations of bacterial extracts were titrated for individual strains and secondary antibodies were titrated to ensure that the assay was not saturated.

Statistical analysis

Statistical analyses were performed using SPSS version 21 software. The Kruskall-Wallis test was used to compare the patient groups and Mann-Whitney (MW) or Dunn's post-test for pairwise comparisons within the patient groups. A chi-squared or Fisher's Exact Test was used to compare non-continuous data. For correlation analyses a Spearman test was used.

The patient groups were a convenience sample ie as many patients were recruited as possible over the period of the study without any prior power calculation being performed. However, using the sample sizes of 39, 36 and 23 for the pSS, RA and OA groups in this study, there is at least 80% power to detect a difference between proportions of 30% and 70% in two groups (assuming a 5% significance level, i.e. no adjustment for multiple comparisons, and a two-tailed test). The comparison of the two largest groups has at least 80% power to detect a difference between proportions of 33% and 67% (see discussion).

RESULTS

Patients

Patient demographics are set out in Table 1.

64 patients with pSS who fulfilled the American-European Consensus Group classification criteria (Vitali et al 2002) agreed to participate in the bacterial antibody reactivity study. 39 of these patients agreed to be assessed for their periodontal status, 6 others were edentulous and the remainder declined or were unavailable.

47 female patients with RA who fulfilled the 1987 American College of Rheumatology criteria agreed to participate in the bacterial reactivity study. 36 agreed to be evaluated for their periodontal status, 1 was edentulous and the remainder declined/were unavailable.

A total of 35 female participants with OA were recruited. 28 of these took part in the bacterial reactivity study and 23 agreed to be assessed for their periodontal status.

40 patients with periodontitis out of 61 previously recruited to a clinical trial at the University of Birmingham School of Dentistry agreed to allow previously collected serum to be used in this study (Chapple et al 2012). The remaining patients did not respond to the invitation.

Periodontal status

Using the American Academy of Periodontology/Centers for Disease Control (AAP/CDC) definition, 21 of 39 pSS patients had none/mild periodontitis (54%), 14 had moderate (36%) and 4 had severe periodontitis (10%). The corresponding figures for the RA group (n=36) were 13 (36%), 16 (44%) and 7 (19%) and for the OA group (n=23) were 12 (52%), 8 (35%) and 3 (13%), respectively. None of these frequencies reached statistical significance for between group comparisons. Comparing specifically the pSS and OA control group using these proportions, Spearman's rho = 0.026 (95% confidence intervals = -0.27 to 0.23). Most of the RA group were on some form of disease-modifying medication (see Table 1) such that it is not possible to evaluate any effect of these medications on periodontitis prevalence in the RA group. Thirty of the 39 pSS patients evaluated in this part of the study were not on such therapy. Thirteen of these 30 patients had moderate periodontitis (43%) and 3 had severe periodontitis (10%). These frequencies do not differ significantly from the overall numbers in this group.

There were no statistically significant differences between the groups for mean attachment loss or for cumulative probing depth. Mean probing depth for the pSS group (n=39) was 1.37 mm (SD=0.29, range of means 1.05-2.49), for the RA group (n=36) was 1.49 mm (SD=0.45, range of means 0.96-3.10) and for the OA group (n=23) was 1.39 mm (SD=0.72, range of means 1.03-4.58). There was a statistically significant difference between the three groups for mean probing depth (KW p=0.046) with the RA group having a higher mean probing depth compared with the OA group (Dunn's post test adjustment p=0.039).

There was no clear relationship between age and periodontal status across the three patient groups (pSS, RA and OA combined) (non-mild versus moderate-severe) (independent 2-sample t-test p=0.26). Current smokers were more likely to have moderate-severe periodontitis than non-smokers and ex-smokers (Fisher's exact test, p=0.034).

Bacterial ELISA data

The ELISA results for antibodies in sera against the 10 bacteria studied in the four patient groups are set out in Figure 1. For 4 of the 6 bacteria associated with periodontitis that we investigated, significant differences in antibody levels were identified between patient groups using the Kruskal-Wallis test.

Two of the bacteria, *A. actinomycetemcomitans* and *P. intermedia,* demonstrated a significant difference across the groups (KW p = 0.037 and p = 0.041 respectively) but without significant differences on pairwise testing (Dunn's post-test p = NS).

P. denticola was significantly different across the 4 groups (KW p = 0.012) with the OA group having lower titres than the three 'inflammatory' groups (Dunn's post-test p=0.046 OA versus pSS; p=0.012 OA versus RA; p=0.034 OA versus periodontitis).

C. showae had higher ELISA titres in the periodontitis group than the pSS or RA groups (KW p = 0.004; Dunn's post test p =0.003 pSS versus periodontitis; p=0.041 RA versus periodontitis). No statistically significant differences were observed between groups for *P. gingivalis, C. gingivalis, E. faecalis*, *F. nucleatum, S. sanguis,* or *S. mutans*.

There was a significant correlation between age and bacterial ELISA levels across the three patient groups (pSS, RA and OA combined) for *A. actinomycetemcomitans* (Spearman's rho p=0.01), *E. faecalis* (p=0.04) and *S. mutans* (p=0.047).

Examining the potential effects of periodontal status across these three groups (pSS, RA and OA combined), bacterial ELISA levels were higher in patients with moderate/severe periodontitis for *P. gingivalis* (Mann-Whitney U-test p=0.034) and *P. intermedia* (Mann-Whitney U test p=0.047).

Examining the potential effects of smoking status across these three groups (pSS, RA and OA combined), bacterial ELISA levels were higher in current smokers for *E. faecalis* (Mann-Whitney U-test p=0.044) and *P. intermedia* (Mann-Whitney U test p=0.031).

If current smokers (n=12) are excluded there is still a correlation between bacterial ELISA levels and age for *A. actinomycetemcomitans* (Spearman's rho p=0.009) and for periodontal status with *P. gingivalis* (Mann-Whitney U-test p=0.043). Excluding current smokers, antibody levels against *P. Denticola* are significantly raised in the RA group compared to the OA group (Dunn's post test p=0.033) and a trend remains for the pSS group compared to the OA group (p=0.061) but this does not reach the p=0.05 threshold for statistical significance.

DISCUSSION

Previous studies have not produced a clear answer to the question of whether the prevalence of periodontitis is higher in pSS than in the general population. Some of these studies have suggested that individual components of the periodontal assessment are abnormal in Sjögren's syndrome (Najera et al 1997, Celenligil et al 1998, Antoniazzi et al 2009), whereas other studies have found no increase in periodontitis (Boutsi et al 2000, Schiødt et al 2001, Kuru et al 2002, Pers et al 2005). We set out to clarify this issue. Our study did not find any increase in the prevalence of periodontitis in pSS compared to the RA or OA groups. In fact, the proportions of patients with mild, moderate or severe periodontits were virtually identical between the pSS and OA groups (46% of pSS patients and 48% of OA patients had moderate or severe periodontitis).

In RA, by contrast, it appears well established that the prevalence of periodontitis is increased in both early and established RA (e.g. Kassebaum et al 2014, Mikuls et al 2014, de Smit et al 2012, de Pablo et al 2009) and linked to RA disease activity (Mikuls et al 2014, de Smit et al 2012). In our study 64% of the RA group had moderate/severe periodontitis. Although this apparently higher prevalence did not reach statistical significance compared to the other groups this study was underpowered to detect a significant difference at an 80% level. This finding is consistent, however, with other studies (de Smit et al 2012). Mean probing depth was significantly greater in the RA group compared to OA controls supporting in general these findings in RA.

One complexity in comparing different studies is the use of different approaches to examining and defining periodontitis. Another is that oral hygiene, age and smoking status are also likely to play a major role in determining levels of periodontitis that may have a greater impact than the presence or absence of sicca/Sjögren's syndrome. We specifically assessed a convenience sample of patients in a secondary care (specialist) environment rather than a primary care population. This represents the 'real-life' pSS population with clinically significant disease whose pathogenesis we wish to better understand. The OA group were recruited as a 'non-inflammatory' group (in terms of inflammatory rheumatic disease) representing the nearest to 'healthy controls' in secondary care. The periodontitis 'positive' control group were selected based on convenient access to existing serum samples but are a selected group (e.g. all are non-smokers) according to the clinical trial entry criteria. Furthermore, although the numbers of participants are comparable to, or larger than, previous studies; it is still a relatively small study. None of the p values would have achieved significance levels had a Bonferroni correction been applied. Nevertheless, our view is that this study demonstrates that there is no clear-cut increased prevalence of periodontitis in pSS that is greater in magnitude than in RA or OA.

This study also set out to address a related question in the same patient population i.e. whether antibody responses to particular oral/periodontal bacteria are increased in the serum of pSS patients compared to controls. The premise is that a higher anti-bacterial antibody response could identify bacteria involved in the pathogenesis of the condition. In the absence of a higher prevalence of periodontitis in the pSS group this hypothesis is now modified to be independent of periodontal status, although our particular *a priori* interest, however, was to study oral bacteria linked to periodontitis.

Although there has been previous interest in categorising differences in oral microbial flora in pSS, any differences compared with controls have principally been viewed as a consequence of xerostomia rather than methodically investigated as a potential cause of, or triggering factor for, pSS. A previous study of 17 Sjögren's syndrome patients and 14 healthy subjects, examined anti-bacterial antibodies, as we have done. Serum antibodies against *A. actinomycetemcomitans* and *P. gingivalis* in patients with pSS were significantly raised compared with controls (Celenligil et al 1998). Other studies have investigated the plaque biofilm to examine which bacterial species can be cultured (Almstahl et al 2001, Kuru et al 2002). Another approach examining T-cell reactivity to Ro60 peptides identified molecular mimicry with *Capnocytophaga ochracea* (Szymula et al 2014).

In this study, we examined antibody responses to 10 bacteria, 6 of whom can be associated with periodontitis. We did not identify any Sjögren's-specific antibody responses. There were, however, subtle differences for 4 of the 6 periodontal bacteria among the patient groups, i.e., *A. actinomycetemcomitans, P. intermedia, P. denticola* and *C. showae*. Whilst we observed a trend towards an association between RA and serum antibodies to *P. gingivalis* as reported previously (de Smit et al 2012), this did not reach statistical significance. Interestingly, in a study of cardiovascular risk and periodontitis, the association of *P. gingivalis* specific antibody levels with periodontal features varied between different strains of *P. gingivalis* (Bohnstedt et al 2010). It is therefore possible that this might be the case in RA or pSS as well. It is important to recognize also that

demographic factors such as age, sex, smoking, ethnicity, dentate and periodontal status all have potential effects on levels of serum antibodies to periodontal bacteria. This has been shown in a large epidemiological study of 8153 participants in the USA (Vlachojannis et al 2010). This demonstrated, among other findings, positive associations between antibody levels to *P. gingivalis* and periodontitis and an increased prevalence of antibodies to *A. actinomycetemcomitans* in the age range 55-69 years compared to youger participants. These findings are pertinent to our data. They also found that current smokers were significantly less likely to exhibit high titres to multiple bacteria than never smokers although this was not the case for all bacteria. In our study we identify some additional associations with these demographic factors for *P. intermedia*, *C. showae, S. mutans* and *E. faecalis* but because of the small size of our study would not wish to overinterpret this data.

Of particular interest to us, however, was that antibodies to *P. denticola* were higher in the 'inflammatory' (pSS, RA and periodontitis) groups than in the non-inflammatory OA group.

The recent development of next generation high-throughput microbial genomic sequencing techniques is opening up alternative approaches to studying the relationship between bacteria and disease and, consequently, new approaches to identifying potential triggering factors in rheumatic disorders (e.g. Scher *et al* 2012, Scher *et al* 2013).

Although we recognize that the data in our study on *P. denticola* is not definitive, nor Sjögren's-specific, it is of potential interest that *Prevotella species* in the oral microbiome have been proposed to be linked to the onset of rheumatoid arthritis (Scher *et al* 2012) and a particular *Prevotella* species, *P. copri*, has been identified, by the same research group, in the gut microbiome of patients with early RA (Scher *et al* 2013). Other studies have also identified changes in the microbiome in RA (Zhang et al 2015). We await with interest for similar studies in pSS to be published.

Therefore, we propose that despite the relatively limited positive data in this study, it demonstrates that there may still be some potential role for studies evaluating serum antibody responses to bacteria as a 'supportive' adjunct to newer techniques of microbiome analysis. It is likely, however, given our experience and that of other studies reported here, that 'non-presumptive' microbiome analysis will be a more powerful screening tool to identify potential bacterial triggers than starting with targeted anti-bacterial antibody responses.

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Table 1. Patient demographics

	pSS (n=64)	RA (n=47)	OA (35)	Periodontitis (n=40)
Females	63	47	35	27
Age (years)				
Mean	61.1	59.1	58.6	52.9
Median	63	61	61	52
SD	10.7	10.7	9.3	8.1
Range	35-80	31-78	45-78	33-73

Antibodies	Ro+La+ n=43	Anti-CCP+ n=46	-	-
	Ro+ La- n=14	RF+ n=42		
	Ro- La+ n=1	RF+ CCP- n=1		
	Negative n=6			
Smoking				
Current	2	8	2	0
Ex-smoker	22	17	17	)
Never	40	22	16	) 40
Medication				
HCQ	6	2	-	-
Pred &/or DMARD	8 (AZA)	29		
HCQ+AZA	1	0		
Biologic	0	10		

SD=standard deviation, RF=rheumatoid factor, CCP = cyclic citrullinated peptide antibodies, HCQ=hydroxychloroquine, DMARD=disease-modifying anti-rheumatic drug, AZA=azathioprine

Figure Legend:

Figure 1. Detection of antibodies to oral bacteria in sera of patients with pSS (n=64), RA (n=47), OA (n=28) and periodontitis (n=40). Statistical analysis was performed using the Kruskal-Wallis test (overarching bar) and Dunn's post test (underlying bars); *p<0.05, **p<0.01. Results are presented as the ELISA optical density normalized for a standard serum.