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**Anti-*Porphyromonas gingivalis* antibodies titres are associated with non-smoking status in early rheumatoid arthritis: Results from the ESPOIR cohort**

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## **ABSTRACT (250 words)**

### **Objectives**

To investigate the possible link between *Porphyromonas gingivalis* (*P. gingivalis*) infection and RA, according to antibody profile, genetic and environmental factors, and RA severity.

### **Patients and Methods**

For assessing *P. gingivalis* infection, serum levels of antibodies directed against *P. gingivalis* LPS were measured in 694 early-RA patients not exposed to steroid or DMARD. Anti-*P. gingivalis* antibodies titers were compared between early-RA patients and various control groups, and according to various patients characteristics.

### **Results**

The titre of anti-*P. gingivalis* antibodies did not significantly differ between RA and controls. Anti-*P. gingivalis* antibody titres did not significantly differ with ACPA, RF, or HLA-shared epitope status. Anti-*P. gingivalis* antibody titres were significantly higher among never smoker patients compared to ever-smoker ( $p= 0.0049$ ). Among non-smokers, high anti-*P. gingivalis* antibody levels were associated with an higher prevalence of erosive change (mSHS erosion subscale  $\geq 1$  : 47.5 vs. 33.3%,  $p=0.0135$ ).

### **Conclusion**

In this large early-RA cohort, we did not detect any association of anti-*P. gingivalis* antibodies with RA or with ACPA status. These results suggest that the association of periodontitis and RA could be linked to other bacterial species than *P. gingivalis* or to another mechanism than citrullination. Nevertheless we found higher anti-*P. gingivalis* antibody titres in non-smokers. In addition, in this population of non-smokers, high anti-*P. gingivalis* antibody titres were associated with a more severe disease. We hypothesize that the role of tobacco in RA pathogenesis is so high that the effect of *P. gingivalis* could be revealed only in a population not exposed to tobacco.

Rheumatoid arthritis (RA) is a systemic, inflammatory autoimmune disorder of unknown, complex and multifactorial aetiology. Its pathophysiology relies on an interaction between gene and environment that triggers auto-immunity. One of the most striking examples is the interaction between tobacco and shared epitope carrying in anti-citrullinated peptide antibody (ACPA) positive RA [1]. In this model, tobacco acts as an external agent inducing citrullination of proteins in the lung that triggers auto-immunity and ACPA production in genetically predisposed subjects, leading to emergence of RA.

Among the other potential environmental factors triggering auto-immunity is the oral microbiome. Several epidemiological studies have suggested a link between periodontal disease and RA. The prevalence of periodontal disease is two-fold increased among patients with RA compared to the general population[2-4], and even more in non-smoking RA patients with a 4-fold increased prevalence[5]. In addition, several cohorts have shown that the risk of development of RA was increased in subjects with periodontal disease [6-8]. This risk was even more important in non-smokers [4, 8].

One of the main bacteria implicated in chronic periodontal disease is *Porphyromonas (P) gingivalis* [9], a microorganism of the oral cavity located in the sub-gingival tissue. *P. gingivalis* has been incriminated in RA pathogenesis because it is one of the few microorganisms with a deiminase capable of transforming arginine into citrulline, and is thus suspected of playing a possible role in the production of ACPA. Some recent studies suggest that the presence of *P. gingivalis* could be associated with development of RA [10-13] and particularly ACPA-positive RA [10, 11, 13-15]. Nevertheless, these results remain controversial [4, 16] and were obtained for most of them from small cohorts.

The objective of this study was to investigate the relationship between *P. gingivalis* infection (as measured by serum levels of anti-*P. gingivalis* antibodies) and RA. This study also aimed to investigate the possible link between *P. gingivalis* infection and RA antibody profile (principally ACPA status), genetic and environmental factors and structural damage in a large cohort of early arthritis patients.

## **PATIENTS AND METHODS**

### **Patients**

The Evaluation et Suivi des POLyarthrites Indifférenciées Récentes (ESPOIR) is a prospective French cohort that included 813 patients with early arthritis. The methodology and the main characteristics of the patients from the ESPOIR cohort have been previously described [17]. The patients were recruited if they had inflammatory arthritis of at least 2 swollen joints lasting for 6 weeks to 6 months and with potential to evolve into RA. Patients were included if they had not received disease-modifying anti-rheumatic drugs (DMARDs) (except within the 15 days before inclusion in the cohort for DMARDs exclusively) or steroids. Patients were excluded if the referring physician considered another defined inflammatory rheumatic disease than RA. The patients were included between December 2002 and March 2005. They have been followed every 6 months during the first 2 years, and every year thereafter. The follow-up is scheduled for at least 15 years from December 2002. Among them, 694 fulfilled the 2010 ACR/EULAR criteria after 2 years of follow-up. Fourteen regional centres in France participated in patients' inclusion.

### ***Clinical and biological assessment***

Clinical variables included total joint count for tenderness and swelling, the Disease Activity Score in 28-joints (DAS28)[18] and the Health Assessment Questionnaire (HAQ)[19]. Laboratory variables included erythrocyte sedimentation rate (ESR, mm/h), C-reactive protein (CRP) level, IgM and IgA rheumatoid factor (RF) (both enzyme-linked immunosorbent assay [ELISA; Menarini], both positive if > 9 UI/ml), ACPA (anti-CCP2, ELISA, DiaSorin, France; positive if > 50 U/ml). All the subjects were genotyped for the HLA-DRB1 shared epitope (SE).

Pro-inflammatory cytokines (Interleukin (IL)-1 $\beta$ , IL-1 receptor antagonist (IL1-Ra), IL-2, IL-4, IL-6, IL-10, IL-17, MCP-1, Tumor Necrosis Factor (TNF)  $\alpha$  and interferon (IFN)  $\gamma$  were previously quantified in ESPOIR cohort at baseline using a commercially available multiplex bead immunoassay, based on the Luminex platform (Fluorokine MAP Multiplex Human Cytokine Panel, R&D Systems, Minneapolis, Minnesota, USA) as previously described [20]. The corresponding quantifications are expressed in ng/ml. The method of quantification

of serum markers of B cell activation ( $\beta$ 2-microglobulin, IgG, IgA, IgM, Free light chain of immunoglobulins [FLCs]) were previously reported [21].

### ***Smoking history***

Patients' smoking habits were evaluated at inclusion. Current smokers were those reporting active smoking. Past-smokers were all patients who had stopped smoking before the first examination at inclusion. Ever-smoker included both current and past-smokers. Non-smokers reported no history of smoking at any time.

### ***Structural assessment***

Patients had radiological evaluation every 6 months during the first two years of follow-up allowing studying parameters associated with structural progression. Radiographs of the hands and feet (antero-posterior views) were collected in the radiography coordinating centre. A standardized reading was performed on X-rays obtained at baseline, 1 year and 2 years of follow-up. All sets of X-rays were read by a trained investigator blinded to clinical evaluation (GT) according to the van der Heijde-modified total Sharp score (mShS). Intra-reader and inter reader reliability (GT and VD-P) were excellent (Intraclass correlation coefficient = 0.97 and 0.93, respectively). The smallest detectable change (SDC) was 1 [22].

Structural damage at inclusion was assessed qualitatively by the presence of typical RA erosions according to their location and aspect, and was rated according to mShS[23]. Radiological progression was assessed between baseline and the end of the second year of follow-up. Radiological progression was defined as a progression of more than the SDC ( $\geq 1$  point of mShS) at 2 years. Rapid Radiological Progression (RRP) was defined as an increase of mShS  $> 10$  within the first two years of follow-up (i.e. 5 points per year).

### **Controls**

#### ***Healthy controls***

Variété is a transversal, non-interventional French national cohort, based on healthy volunteers for establishing normative data for IGF-I and other hormones in the general population (ClinicalTrials.gov identifier: NCT01831648). A total of 974 healthy subjects have been recruited in 10 centres all around France. Subjects with medical conditions and medications that may affect IGF-I measurement have been excluded. A random sample of 79 healthy controls from the Variété cohort was matched, for age- and gender, to a representative sample of 79 RA patients from ESPOIR cohort. Since this cohort focused on healthy subjects with no risk factors, it included only non-current smokers: among them, 83.7% were never-smokers and the remaining 16.2% did not smoke more than 10 cigarettes/day.

### ***Sicca Controls***

Sicca controls were 54 patients from the cohort of patients with sicca symptoms referred to the Rheumatology Department of the Bicêtre Hospital for a diagnostic procedure to assess if patients have Sjögren's syndrome. To be considered as controls patients, these subjects were excluded if they had auto-antibodies, lymphocytic sialadenitis on salivary gland biopsy or any features of auto-immunity. Nevertheless, some of them had objective dryness features. These controls were age- and gender-matched to a random sample of RA patients from ESPOIR cohort. Their smoking status was known as never or ever smoker, with no mention of current or past smoking status. This group included 19/51 (37.3%) ever smokers.

### ***Periodontitis controls***

The last control group consisted in 61 patients with clinically proven severe periodontitis. In this group, 21 (34.5%) patients were non-smokers, 40 (65.6%) were ever smokers, including 13 (21.3%) current smokers. These patients were not matched with ESPOIR cohort patients, and included a higher proportion of males. We therefore took a matched subsample of this group having a same proportion of male as the RA cohort to perform sensitivity analyses for comparisons with early RA patients. In addition, for this group of patient, Micro-Indent test was performed to detect the presence of *P. gingivalis* in periodontal tissue (see below).



## **Bacterial serologic measurement and detection of *P. gingivalis***

### ***Anti-*P. gingivalis* antibodies measurement***

Immunoglobulin G antibodies specific to LPS of *P. gingivalis* were measured using a homemade ELISA. The wells of 96-well flat-bottom microtiter plates were coated in triplicate with LPS of *P. gingivalis*. After washing and blocking the plates, serum samples were added to individual wells and specific human IgG antibodies were detected with an alkaline phosphatase-conjugated antihuman immunoglobulin. The absorbance was read at 405 nm using an ELISA plate reader. The results were expressed as an ELISA index (EI), which was the mean OD 405 nm of a given serum divided by the mean OD 405 nm of the calibrator (reference serum) [11].

### ***Bacteria identification (DNA-DNA hybridation)***

Bacteria identification was made in the gingival pockets, with the kit Micro-Ident according to the manufacturer's instructions (Hain Lifescience, Germany), as follows. DNA–DNA hybridization is a molecular biology technique that measures the degree of genetic similarity between pools of DNA sequences; it can be used to identify different species in a pluribacterial sample. In our case DNA was isolated from gingival pockets, amplified with specific primers (supplied in the kit; HainLifescience, Germany). In the next step, the amplicons were chemically denatured, since detection on the strip was done using single-stranded DNA. The strip was coated with highly specific probes which were complementary to selectively amplified nucleic acid sequences. The single-stranded amplicon binds specifically to the analog probes during hybridization, while non-specifically bound amplicons were removed in subsequent washing steps. During the conjugate reaction, the specifically bound amplicon was marked with the enzyme alkaline phosphatase and was then made visible in a colorimetric detection reaction.

## **Ethics**

The protocols of the ESPOIR cohort study and of the Variété cohort study were approved by the Ethics Committees of Montpellier University Hospital, and Hôpitaux Paris-Sud, France, respectively. All patients from ESPOIR and Variété Cohorts as well as sicca controls included in the present study gave their written informed consent.

### **Statistical analyses**

All analyses were restricted to the subgroup of 694 patients fulfilling the ACR/EULAR criteria. Categorical variables are reported as numbers (percentages) and were compared using a  $\chi^2$  or, when appropriate, Fischer's exact test. Quantitative variables are reported as mean ( $\pm$  standard deviation [SD]) and were compared using a Student t-test. For correlations analyses, Spearman's correlation coefficients were obtained.

Since results were not normally distributed, anti-*P. gingivalis* antibody titres were log-transformed. Also, anti-*P. gingivalis* titres were used as dichotomous variable. For that purpose, high titres were defined as a titre above the 75th percentile (upper quartile) of distribution in the RA patients of the ESPOIR cohort.

To assess if anti-*P. gingivalis* antibodies titres were associated with early RA, titres were compared between RA patients and the different control groups.

To identify parameters associated with anti-*P. gingivalis* antibodies titres, their serum levels were compared according various demographic, clinical characteristics, biological/biochemical and radiological variables (for categorical variables) and correlated with the levels of clinical, biological and radiological variables (for quantitative parameters).

For all analyses, a  $p < 0.05$  was considered statistically significant. Confidence intervals were calculated at the 95% level. Statistical analyses involved use of SAS 9.3 (SAS Inst., Cary, NC).

## **RESULTS**

### **Patients with early RA and controls**

Among patients from the ESPOIR cohort, 694 fulfilled the ACR/EULAR criteria for RA after 2 years of follow-up. Their main characteristics are presented in Table 1. Age, sex and smoking status of early RA and controls groups are reported in Table 2.

### **Anti-*P. gingivalis* antibody titres among controls and early RA patients**

Anti-*P. gingivalis* antibody titres did not significantly differ between early RA patients and healthy controls ( $1.47 \pm 0.42$  vs.  $1.49 \pm 0.33$ ,  $p=0.66$ ), sicca controls ( $1.47 \pm 0.42$  vs.  $1.51 \pm 0.47$ ,  $p=0.53$ ) or periodontitis controls ( $1.47 \pm 0.42$  vs.  $1.55 \pm 0.41$ ,  $p=0.17$ ) (figure 1). Among periodontitis controls, the level of anti-*P. gingivalis* antibodies tended to be higher among females but this did not reach significance ( $1.54 \pm 0.35$  vs.  $1.62 \pm 0.47$ ,  $p=0.28$ ). We therefore compared the level of anti-*P. gingivalis* antibodies between early RA and the sex-matched subsample of periodontitis controls and found a trend to an higher level in periodontitis controls ( $1.47 \pm 0.42$  vs.  $1.60 \pm 0.44$ ,  $p=0.12$ ). In addition, among periodontitis controls, 24 (39.34%) had *P. gingivalis* detected in periodontal tissue, these patients had a higher level of *P. gingivalis* antibodies, compared to periodontitis patients for whom *P. gingivalis* was not detected (1.74 vs. 1.43;  $p=0.008$ , figure 2). Thus, a high titre of anti-*P. gingivalis* is a good indirect marker of chronic *P. gingivalis* infection.

### **Anti-*P. gingivalis* antibody titres among different subsets of early RA patients**

Anti-*P. gingivalis* antibody titres did not significantly differ with ACPA, RF, or HLA- shared epitope status (figure 3). Among ACPA positive patients, the level of anti-*P. gingivalis* antibody titres did not correlate with the level of ACPA ( $\rho=0.042$ ,  $p=0.46$ ), or RF titres ( $\rho=0.013$ ,  $p=0.82$ ). Also, they did not correlate with disease activity (DAS28 or its components), pro-inflammatory cytokine levels, serum levels of B cell activation markers ( $\beta$ 2-microglobulin, IgG, IgA, IgM, FLCs) or structural damage as measured by the total mShS and its subscales. However, patients with high anti-*P.gingivalis* antibody levels were more likely to have typical erosion related to RA than those with lower values (35/164 [21.34%] vs. 69/497 [13.88%],  $p=0.02$ ). Also, 40.1% (65/162) of the patients with high anti-*P.gingivalis*

antibody titres had erosive RA (as measured by an mShS erosion subscale  $\geq 1$ ) compared to 34.9% (173/496) of the patients with lower titres ( $p=0.23$ , figure 4).

### **Higher anti-*P. gingivalis* antibody titres in never smokers**

Anti-*P. gingivalis* antibody titres were significantly lower among ever-smoker patients compared to never smoker ( $1.42 \pm 0.43$  vs.  $1.51 \pm 0.43$ ,  $p= 0.0049$ ) and particularly in current smoker patients compared to never smoker ( $1.37 \pm 0.42$  vs.  $1.51 \pm 0.43$ ,  $p= 0.0003$ ), but not past-smokers ( $1.51 \pm 0.43$  vs.  $1.47 \pm 0.43$ ,  $p= 0.28$ ). Also, patients with high anti-*P. gingivalis* antibody titres were more likely to be non-smoker than those with low titres (107/175 [61.1%] vs. 254/265 [48.9%],  $p=0.005$ ).

In the control groups, there was a numerical, but not significant, higher level of anti-*P. gingivalis* antibodies in never smokers compared to ever smokers in the sicca group ( $1.56 \pm 0.47$  vs.  $1.46 \pm 0.48$ ,  $p=0.45$ ), and compared to current smokers in the periodontitis group ( $1.57 \pm 0.48$  vs.  $1.48 \pm 0.33$ ,  $p=0.54$ ).

Among RA patients, smoking status did not alter the level of other antibodies (anti-CCP, IgM-RF, FLC, total IgG, IgA, IgM), except the level of IgA-RF that were found significantly higher in smokers than in non smokers ( $45.6 \text{ UI} \pm 107.5$  vs  $29.1 \pm 60.4$ ;  $p=0.014$ ).

### **Higher anti-*P. gingivalis* antibody titres in non-smokers were associated with a more severe disease**

Among non-smokers, patients with high anti-*P. gingivalis* antibody levels were more likely to have erosive disease than those with lower titres as demonstrated by a higher proportion of patients having typical erosion related to RA (22/102 [21.6%] vs. 32/241 [13.3%],  $p=0.054$ ) and having a mShS erosion subscale  $\geq 1$  at baseline (48/101 [47.5%] vs. 80/240 [33.3%],  $p=0.014$ ). This trend was not observed in the ever smoker group, neither past nor current smokers (figure 5). In the current smoker group, we observe an inverse but not significant

relationship ( $p=0.20$ ). However, anti-*P. gingivalis* antibodies titres were not predictive of structural progression, whatever the definition used.

## DISCUSSION

This study is one of the largest that examined the possible link between *P. gingivalis* infection and RA, with a particular focus on RA-antibody profile (principally ACPA status), genetic and environmental risk factors and structural damage. In this cohort of early RA patients, we could not detect any association between anti-*P. gingivalis* antibodies and RA whatever the ACPA status. Nevertheless, we found that anti-*P. gingivalis* antibody titres were increased in never smokers. In this group of never smoker patients, increased levels of anti-*P. gingivalis* antibody were associated with a more erosive disease.

We did not find higher anti-*P. gingivalis* antibody titres in RA patients compared to non-RA controls. There is to date no consensual method for dosing anti-*P. gingivalis* antibodies and each of them may be questionable. We here used an assay that detected antibodies to *P. gingivalis* LPS [16], rather than lysate or purified proteins used in some previous studies [10, 14, 15]. A method similar to that we used demonstrated such association with RA in a previous study [11]. Moreover, we found higher antibody titres in our positive control group, i.e. patients with *P. gingivalis*-associated periodontitis. Thus, our method seems to be valid for assessing a long-term exposure to *P. gingivalis*. Nevertheless, as shown by Scher *et al*, the presence of anti-*P. gingivalis* antibody is not necessarily associated with the presence of a currently active *P. gingivalis* infection. Therefore, none of these methods, including our, is able to detect current *P. gingivalis* infection, but they assess prior exposure to this pathogen. The only method able to prove *P. gingivalis* infection is the direct detection of the bacteria. Unfortunately, we did not have the opportunity to assess this in the ESPOIR cohort. At the time of the conception of this cohort, the implication of oral microbiome in RA pathogenesis was not suspected yet. Nevertheless, it is the largest cohort, involving almost 700 patients, exploring exposure to *P. gingivalis* in early RA. Compared with most of the previous studies, we here included early RA patients and not long standing established RA. Results of the previous studies assessing *P. gingivalis* infection in early RA are controversial [4, 13] and two

recent studies did not also find any association between anti-*P. gingivalis* antibodies and RA [4, 16], particularly in the absence of proven periodontitis[16].

This study did not refute the potential role of oral microbiome in RA. First, the association between periodontitis and RA found in many studies, and confirmed in a recent systematic review [24], may be linked to another pathogen than *P. gingivalis* as recently suggested by Scher et al [4]. Second, tobacco plays such a major role in triggering RA that it may overtake and mask the potential role of *P. gingivalis* in smoker population. The role of *P. gingivalis* and other oral bacterial species could be revealed only in a population not exposed to tobacco. Effectively, the increased prevalence of periodontitis in non-smoker RA patients suggested in 2 previous studies, underlined that the link between periodontitis, oral microbiome and RA could be more pronounced in the non-smoking population [4, 8]. Last, even if *P. gingivalis* plays a major role, it may be by another mechanism than inducing citrullination since in number of studies including ours, there is no difference of anti-*P. gingivalis* titres in patients with or without ACPA. It could be also linked to the use of an anti-CCP2 kit that detects IgG but not IgA isotypes, the latter being possibly more associated to mucosa-associated antibody production. Nevertheless, other reports which found an association between *P. gingivalis* and ACPA status used similar assays [10, 15]. Although controversial [25], it has been recently found that auto-citrullination of *P. gingivalis* peptidylarginine deiminase (PPAD), is probably not the underlying mechanism linking *P. gingivalis*-associated periodontitis and RA [26]. Moreover, in 2 recent studies, it has been found that patients with periodontitis or with pre symptomatic RA have a higher titre of antibodies against uncitrullinated antigens suggesting that breaking of tolerance to uncitrullinated peptides could be the first event in these patients [27, 28] . Even more interestingly, in patients with periodontitis, the titres of antibodies against uncitrullinated antigens were more elevated in non-smokers than in smokers suggesting that the non-citrulline specific breaking of tolerance associated with periodontitis was even more important in non-smokers [28]. Also, in RA patients with periodontitis, smoking status did not influence ACPA titres, but had an impact on anti-*P. gingivalis* titres that were found lower in smokers than in non-smokers [29]. This observation clearly fits with our findings of a possible link between *P. gingivalis* infection and RA and severity of RA only in non-smokers. An alternative hypothesis would be

a protective effect of smoking specifically on anti-*P. gingivalis* antibody generation, without any general effect on antibody or auto-antibody generation.

In conclusion, we did not detect any association between anti-*P. gingivalis* antibodies and RA or ACPA status in this large cohort of early-RA. These results suggest that the association of periodontitis and RA could be linked to other bacterial species than *P. gingivalis* or to another mechanism than citrullination. Nevertheless we found higher anti-*P. gingivalis* antibody titres in non-smokers compared to smokers. In addition, in this population of non-smokers, high anti-*P. gingivalis* antibody titres were associated with a more severe disease. We hypothesize that the role of tobacco in RA pathogenesis is so high that the role of *P. gingivalis* could be revealed only in a population not exposed to tobacco. Alternatively, smoking might decrease anti-*P. gingivalis* immune response. Finally, our findings suggest that the association of periodontitis and RA could be linked to other bacterial species than *P. gingivalis* or to another mechanism than citrullination and reinforce the importance of investigating the potential interest of oral hygiene and supragingival scaling in RA patients, as suggested in a recent meta-analysis [30].



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**Competing interests**

Authors have no competing interest in relation to this study

**Contributorship**

Conception and design: Raphaèle Seror and Xavier Mariette

Data collection: all

Analysis and interpretation: Raphaèle Seror, Xavier Mariette, Sandrine David-Le Gall

Drafting and critical reviewing: all

Final approval: all

**Ethical approval information**

The ESPOIR cohort study was approved in July 2002 by the ethics committee of Montpellier, France (no. 020307), allowing future clinical projects on the database. All patients gave their signed informed consent before inclusion.

**Data sharing statement**

Data set is available on request

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**Table 1. Characteristics of early-RA patients form the ESPOIR cohort**

	RA patients N= 694
Sex (female)	543 (78.2%)
Age (years)	48.5 ± 12.3
Symptom duration (days)	74.8 ± 76.6
BMI (kg/m <sup>2</sup> )	25.1 ± 4.6
Ever smoker	333 (48.0%)
Current smokers	151 (21.8%)
Past smokers	182 (26.2%)
CRP (mg/dl)	22.8 ± 34.7
CRP us (mg/dl)	20.9 ± 33.5
ESR (mm)	29.9 ± 24.8
PGA (/100mm)	61.7 ± 24.6
Tender joint count (/28)	9.4 ± 7.1
Swollen joint count (/28)	7.9 ± 5.4
DAS28	5.3 ± 1.2
Anti-CCP	315 (45.4%)
IgM-RF	372 (53.6%)
Anti-CCP and/or RF (IgM or IgA)	438 (63.1%)
Typical RA erosive change	181 (32.9%)
Modified Sharp score	5.2 ± 7.4
Current use of DMARD	48 (6.9%)
Current use of oral corticosteroids	0 (0.0%)

Current use of oral NSAIDs	487 (70.2%)
Anti- <i>P. gingivalis</i> antibody titre	3.80 ± 2.23
Anti- <i>P. gingivalis</i> antibody titre (logtransformed)	1.47 ± 0.43

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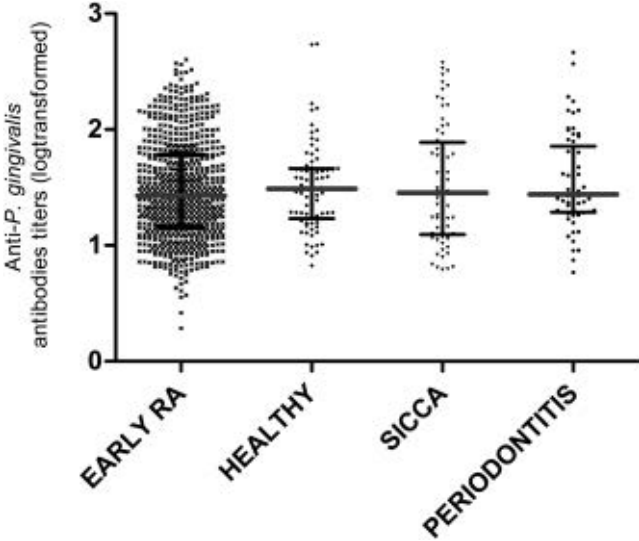


**Table 2. Characteristics of early-RA patients and control groups**

	ESPOIR N=694	HEALTHY N=80	SICCA N=54	PERIODONTITIS N=61
Age (years)	48.5 ± 12.3	47.6 ± 11.9	48.9 ± 11.5	50,7± 8,3
Sexe (female)	543 (78.2%)	66 (84.6%)	46 (85.2%)	25 (41.0 %)
Ever smoker	333 (48.0%)	13 (16.2%)*	19/51 (37.3%)	40 (65.6%)

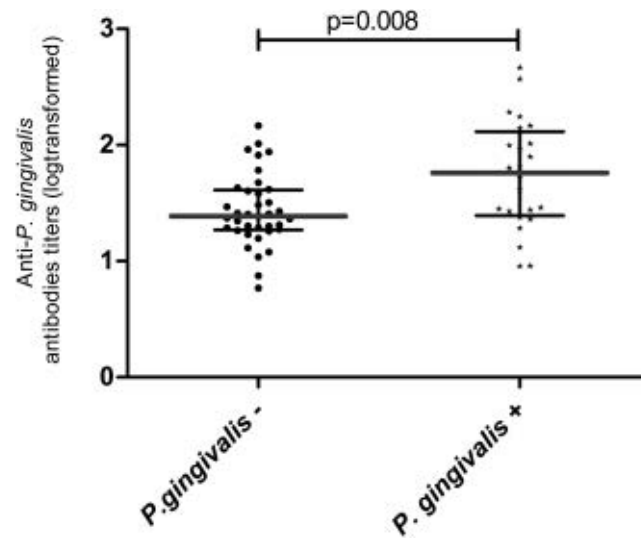
\* All past smokers

Figure 1. Anti-*Porphyromonas gingivalis* antibody titres in early-RA patients and various control groups



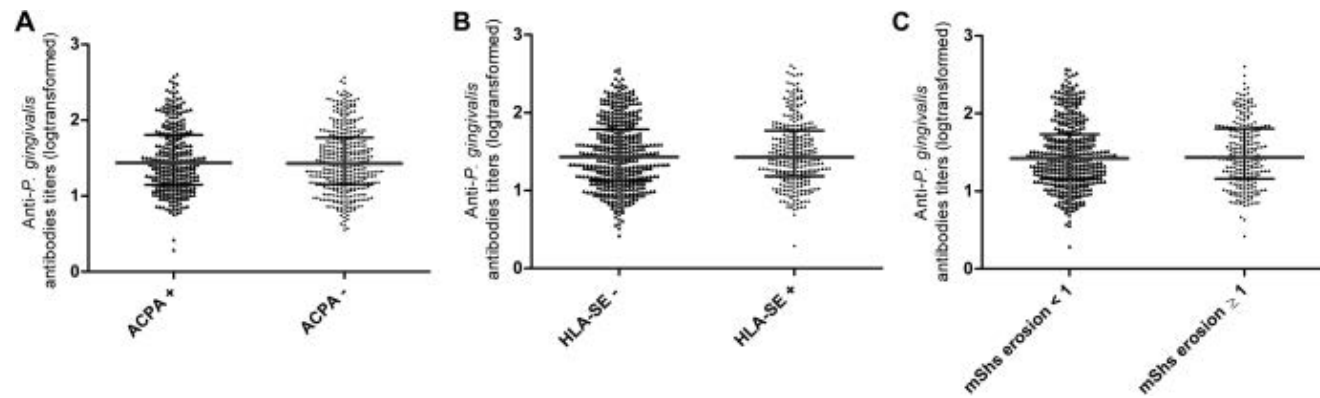
**Figure 2. Anti-*Porphyromonas gingivalis* antibody titres in periodontitis controls according to detection of *Porphyromonas gingivalis* in periodontal tissue**

*P. gingivalis* +: presence of *Porphyromonas gingivalis* in periodontal tissue samples; *P. gingivalis* -: absence of *Porphyromonas gingivalis* in periodontal tissue samples.



**Figure 3. Anti-*Porphyromonas gingivalis* antibody titres according to ACPA (A), HLA-SE (B) and erosive change (C) in early-RA patients**

ACPA: anti-citrullinated peptides antibody; HLA-SE: HLA shared epitope; mShS: van der Heijde-modified total Sharp score



**Figure 4. Link between anti-*Porphyromonas gingivalis* antibody titres, erosive changes according to smoking status in early-RA patients**

**(A):** Comparisons of anti-*Porphyromonas gingivalis* antibody titres according to smoking status

**(B):** Link between anti-*Porphyromonas gingivalis* antibody and presence of erosive changes according to smoking status in early-RA patients. Percentage of patients with erosive RA (defined by an mShS erosion subscale  $\geq 1$ ) in patients with high anti-*P.gingivalis* antibody titres  $\geq 75$ th percentile (dark grey) and in those with low anti-*P. gingivalis* antibody titres (light grey) according to smoking status.

\*:  $p=0,014$

