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Original article

Arthritis autoantibodies in individuals without rheumatoid arthritis: follow-up data from a Dutch population-based cohort (Lifelines)

Johanna Westra¹, Elisabeth Brouwer¹, Elisabeth Raveling-Eelsing¹, Suzanne Arends¹, Amaal Eman Abdulle², Caroline Roozendaal³, Myrthe A. M. van Delft⁴, Rene E. M. Toes⁴, Leendert A. Trouw⁴, Arjan Vissink⁵ and Menke J. de Smit^{1,5}

Abstract

Objectives. To assess whether the presence of arthritis autoantibodies alongside IgG ACPA predicts clinically suspect arthralgia in ACPA-positive subjects without RA.

Methods. In the population-based Lifelines cohort ($n = 40\,136$), 308 IgG ACPA-positive individuals without RA were present. Serum levels of IgA ACPA, IgA and IgM RF, and IgG anti-carbamylated antibodies were measured at baseline. Individuals were divided based on the Connective tissue disease Screening Questionnaire after 2 years follow-up. Antibodies to *Porphyromonas gingivalis* were determined at baseline and related to presence of periodontitis and joint complaints at 2 years follow-up.

Results. Of 308 subjects 53.6% were also seropositive for IgA ACPA, 42.2% for IgM RF, 23.7% for IgA RF and 13.6% for anti-carbamylated antibodies. We defined 75 persons with clinically suspect arthralgia at risk for RA based on CTD Screening Questionnaire at follow-up. Significantly more seropositivity for IgM RF and higher levels of IgG ACPA, IgA ACPA and IgM RF were found in clinically suspect arthralgia compared with no-clinically suspect arthralgia. In multivariate logistic regression correcting for age, gender and never smoking, positivity for three or more extra autoantibodies was significantly associated with clinically suspect arthralgia. Although levels of anti-*P. gingivalis* were not different between groups, they were significantly correlated to levels of both RFs, and both ACPAs in clinically suspect arthralgia.

Conclusions. ACPA-positive individuals without RA who develop clinically suspect arthralgia have more and higher levels of other arthritis autoantibodies at baseline. Levels of anti-*P. gingivalis* are not related to self-reported periodontitis or clinically suspect arthralgia, but are correlated to arthritis autoantibodies in clinically suspect arthralgia.

Key words: arthritis autoantibodies, arthralgia, *P. gingivalis*, periodontitis

Rheumatology key messages

- ACPA-positive persons with clinically suspect arthralgia have more and higher levels of arthritis autoantibodies.
- Seropositivity for multiple arthritis autoantibodies is significantly associated with development of clinically suspect arthralgia.
- Antibodies to *P. gingivalis* are correlated to arthritis autoantibodies in persons with clinically suspect arthralgia.

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Introduction

RA is a chronic autoimmune disease, characterized by inflammation in the joints, causing pain, cartilage and bone damage, and disability [1], and presence of the autoantibodies RF and ACPA are part of the classification criteria for RA [2].

The development of RA is considered a multistep process. EULAR has identified several phases: (i) presence of genetic and environmental risk factors, (ii) systemic autoimmunity associated with RA, (iii) symptoms without clinical arthritis, (iv) unclassified arthritis and finally (v) RA [3]. Genetic and environmental risk factors include shared epitope (HLA-SE) and other genetic loci, smoking, periodontitis and gut dysbiosis, which are discussed by Karlson *et al.* [4]. Regarding the relation between periodontitis and arthritis, much attention has been given to *Porphyromonas gingivalis*, an oral bacterium that possesses a peptidyl arginine deiminase enzyme that is able to citrullinate bacterial and human proteins [5]. Antibodies to *P. gingivalis* have been regarded as a measure for periodontitis, and may be of predictive value for development of RA.

RF and ACPA are present years before clinical diagnosis of RA [6], although <50% of ACPA- and RF-positive arthralgia patients develop RA in a median follow-up time of 28 months [7]. Anti-carbamylated protein antibodies (anti-CarP) are also present in RA [8]. Carbamylation is a posttranslational modification, converting lysines into homocitrullines [9]. Anti-CarP antibodies can also be detected before diagnosis [10] and may also be present in patients seronegative for RF and ACPA [8]. These antibodies may be of additional value in the diagnosis of RA. In a recent literature study [11], it was reported that triple positivity for ACPAs, RFs and anti-CarP antibodies resulted in a higher specificity for RA (98–100%), but with lower sensitivity (11–39%). Anti-CarP antibodies have been detected in arthralgia patients [12]. Another recent publication, however, demonstrated no additional value for RA development of measuring anti-CarP in arthralgia patients in clinical practice, so the data of Verheul *et al.* [11] cannot be extrapolated to the setting of populations with symptoms [13].

In the above-mentioned phases defined by the EULAR, the symptomatic phase preceding clinical arthritis is the first opportunity to recognize patients at risk for progression to RA. This phase is less studied compared with the other phases. Recently, a EULAR taskforce defined the clinical characteristics of patients with arthralgia who are considered at risk for RA, described as clinically suspect arthralgia [14]. These clinical characteristics, of which five are from history-taking and two from physical examination, can be used to establish risk of progression to RA. The history-taking questions involved joint symptoms of recent onset (<1 year), symptoms located in MCP joints, duration of morning stiffness ≥ 60 min, most severe symptoms present in the early morning and presence of a first-degree relative

(FDR) with RA. Physical examination included difficulty of making a fist and positive squeeze test of MCP joints [14].

In a previous study [15], presence of ACPA in Lifelines, a population-based cohort from the northeast of the Netherlands, was investigated. In a large cohort of >40,000 participants, 311 individuals were identified that were ACPA positive (Phadia ACPA-CCP2 levels ≥ 6.2 U/ml), but did not have RA. The aim of the present study was to assess whether presence of arthritis associated autoantibodies next to IgG ACPA can predict clinically suspect arthralgia in ACPA positive subjects without RA. In addition, it was assessed whether levels of anti-*P. gingivalis* are related to self-reported periodontitis and joint complaints or arthritis-associated autoantibodies.

Methods

Study design and study population

Lifelines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviour of 167 729 persons living in the north of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioural, physical and psychological factors that contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics [16]. Information on application and data access procedure is summarized on <http://www.Lifelines.nl>. The Lifelines Cohort Study was conducted according to the principles of the Declaration of Helsinki, approved by the local ethics committee of the University Medical Center Groningen. All participants provided written informed consent. In a previous study [15] cross-sectional data from 40 136 participants were included at baseline visits (2012–13). ACPA detection was performed by measuring IgG anti-CCP2 on the Phadia-250 analyser (ThermoFisher Scientific, Freiburg, Germany) with levels ≥ 6.2 U/ml considered positive. The baseline questionnaire was taken on demographic and clinical information, including smoking, periodontal health and early symptoms of musculoskeletal disorders. RA was defined by a combination of self-reported RA, medication use for the indication of rheumatism and visiting a medical specialist within the last year. Of 40 136 unselected individuals, 401 (1.0%) had an ACPA level ≥ 6.2 U/ml, of which 80 persons (22.4%) had RA (15.2% had defined RA according to the criteria and 7.2% self-reported RA only). In participants without RA (non-RA), 311 persons (0.8% of the total cohort) were ACPA-positive.

For the present study, serum samples were tested from non-RA participants who were IgG ACPA-positive. No serum samples were available from three participants, so 308 persons were included. In the obtained sera, levels of IgA ACPA, IgM and IgA RF, and

IgG anti-CarP were measured, as well as levels of antibodies to *P. gingivalis*.

CTD Screening Questionnaire

Lifelines participants were invited to complete the CTD Screening Questionnaire (CSQ) [17] during the follow-up assessment, which had taken place ~2 years after the baseline assessment. For the 308 non-RA participants this was done in 23 months (median; interquartile range 23–25). The CSQ comprises 30 questions designed at identifying potential CTDs. The RA-related questions were the following: (1) 'Do you have joint stiffness in the morning', (2) 'Do you have joint complaints for >3 months', (3) 'Do you have joint stiffness in the morning lasting at least 1 h for >6 weeks', (4) 'Do you have complaints in the same joints on both sides of your body', (5) 'Do you have joint pain', (6) 'Do you have pain in the wrist or hand', (7) 'Do you have swollen hands or wrists' and (8) 'Is it difficult for you to make a fist'.

Persons that answered 'yes' to one or more of these questions were considered to have joint complaints. Of these, questions 3, 6, 7 and 8 overlap with the EULAR taskforce questions, therefore persons who answered 'yes' to these questions and/or had an FDR with RA reported at baseline were considered clinically suspect for arthralgia at risk for RA (clinically suspect arthralgia) and analysed separately.

Laboratory measurements

IgA ACPA measurements were performed using a modification of the anti-CCP2 kit (Euro Diagnostica, Arnhem, The Netherlands) as described previously [18]. A pool of four high-level IgA ACPA sera served as standard curve expressed in arbitrary units (U/ml). Seropositivity was defined as greater than mean + 2 s.d. of healthy controls, and set to 1 U/ml [18].

Levels of IgG anti-CarP antibodies against carbamylated fetal calf serum were assessed using the protocol as described by Shi *et al.* [8]. Seropositivity was defined as greater than mean + 2 s.d. of a distinctive healthy control cohort from the same study.

IgM and IgA RF levels were assessed using a validated in-house ELISA. Levels were expressed in IU/ml, and seropositivity was defined as ≥ 10 IU/ml for IgM RF and ≥ 25 IU/ml for IgA RF for diagnostic purposes [19]. In a recent publication, IgM RF levels ≥ 3.5 IU/ml were also considered positive [13].

Antibodies to *P. gingivalis* were assessed as described previously [20]. In short, antibodies to *P. gingivalis* were measured by an in-house ELISA, in which a pooled lysate of four randomly selected clinical isolates of *P. gingivalis* from patients with periodontitis was used as antigen. Standard curves were made from protein standards (N protein-standard SL; Siemens Healthcare Diagnostics, den Haag, the Netherlands). Detection of anti-*P. gingivalis* antibodies was carried out with mouse anti-human IgG (Fc fragment specific, clone JDC-10; Southern Biotech, Uithoorn, the Netherlands). Arbitrary

cut-off values for anti-*P. gingivalis* positivity was 5 mg/l, defined as mean values + 2 s.d. of healthy subjects without periodontitis and without cultivable subgingival *P. gingivalis* [healthy control, $n = 36$, mean (s.d.) age 34 (15) years, 53% female, 14% current smoker] as described previously [21].

Statistics

Statistics were performed with The Statistical Product and Service Solutions (SPSS, version 25; IBM Corp., Armonk, NY, USA). Results were expressed as numbers of subjects (percentages), mean (s.d.) or median (interquartile range) for categorical, normally and non-normally distributed data, respectively. χ^2 test, independent samples *t* test, and Mann-Whitney *U* test were used as appropriate to compare characteristics between participants with and without RA. Multivariable logistic regression with clinically suspect arthralgia based on questions related to the EULAR definition (yes/no) as dependent variable was performed to correct the association with the presence of joint complaints and autoantibodies for age, gender and never smoking. *P*-values <0.05 were considered as statistically significant.

Results

Distribution of RA-related autoantibodies

Arthritis-associated autoantibodies were measured in serum samples of 308 IgG ACPA-positive individuals without RA. Of these persons, 166 (53.6%) were also positive for IgA ACPA, 73 (23.7%) for IgA RF, 78 (25.3%) for IgM RF using a cut-off of 10 IU/ml and 130 (42.2%) using a cut-off of 3.5 IU/ml, and 42 (13.6%) for anti-CarP. There was a strong correlation (Spearman ρ between 0.42 and 0.70, all $P < 0.0001$) between all autoantibodies.

From the 308 ACPA-positive individuals, 178 (57.8%) had responded to the CSQ. Baseline characteristics of this group and the group with missing CSQ group are summarized in Table 1. It can be seen from the table that the persons for whom CSQ data were available were older, had lower BMI, were less often smokers and more often had an FDR with rheumatism. However, there were no significant differences between autoantibody positivity or autoantibody levels between these groups.

Evaluation of arthritis autoantibodies and joint complaints

Scores on questions regarding self-reported joint complaints are listed in Table 2. In total, 83 out of 178 persons (46.6%) scored positive for one or more joint complaints. Most participants reported joint pain and joint complaints for >3 months, and around 25% of the persons suffered from morning stiffness and from joint complaints on both sides of their body.

TABLE 1 Baseline information of all ACPA-positive participants without RA, divided by availability of CSQ data at follow up (median 23 months)

	Total group (N = 308)	CSQ available (N = 178)	Missing CSQ (N = 130)	P-value, CSQ yes/no
Age, years, median (IQR)	46 (35–53)	47 (37–53)	44 (33–50)	0.05
Gender (female), n (%)	191 (62.0)	112 (62.9)	79 (60.8)	0.70
BMI, kg/m ² , median (IQR)	25.4 (23.2–27.8)	24.8 (22.9–27.3)	26.0 (23.8–28.7)	0.002
Smoking				
Never smoker, n (%)	124 (940.3)	76 (42.6)	48 (36.9)	0.31
Former smoker, n (%)	107 (34.7)	67 (37.6)	40 (30.8)	0.21
Current smoker, n (%)	77 (25.0)	35 (19.7)	42 (32.3)	0.01
Alcohol (g/day), median (IQR)	3.4 (0–8.6)	3.6 (0–7.4)	2.9 (0–10.8)	0.74
Periodontitis (self-reported), n (%)	44 (14.3)	27 (15.2)	17 (13.1)	0.61
Joint complaints: pain hands and/or feet, n (%)	65 (21.1)	39 (21.9)	26 (20)	0.82
Joint complaints: stiffness in hands and/or feet, n (%)	63 (20.5)	37(20.7)	26 (20)	0.97
FDR with rheumatism, n (%)	47 (15.3)	34 (19.1)	13 (10.0)	0.03
ACPA IgG pos, n (%)	308 (100)	178 (100)	130 (100)	1.00
ACPA IgG level, median (IQR)	16 (9.0–61)	17.5 (9.0–61.5)	14 (9.0–60.5)	0.75
ACPA IgA pos, n (%)	166 (53.9)	97 (54.5)	69 (53.1)	0.85
ACPA IgA level, median (IQR)	1.1 (0.6–2.3)	1.1 (0.6–2.1)	1.1 (0.6–2.4)	0.67
RF IgA pos, n (%)	73 (23.7)	40 (22.5)	33 (25.4)	0.55
RF IgA level median (IQR)	4.5 (1.5–19.2)	4.0 (1.5–18.7)	5.3 (1.4–21.5)	0.59
RF IgM pos (>3.5), n (%)	130 (42.2)	70 (39.3)	60 (46.2)	0.23
RF IgM pos (>10.0), n (%)	78 (25.3)	48 (27.0)	30 (23.1)	0.44
RF IgM level, median (IQR)	2.7 (0.8–10.7)	2.5 (0.8–11.2)	3.0 (0.8–8.4)	0.89
Anti-CarP pos, n (%)	42 (13.6)	25 (14.0)	17 (13.1)	0.81
Anti-CarP level, median (IQR)	101 (44–208)	95 (43–206)	104 (51–212)	0.54

Significant P values are shown in bold. CSQ: CTD Screening Questionnaire; IQR: interquartile range; anti-CarP: anti-carbamylated protein antibody; FDR: first-degree relative; pos: positive.

Next, differences in autoantibody profile were evaluated between persons with and without joint complaints. Participants with joint complaints were significantly older and were more often female (Table 3). Furthermore, IgA RF levels ($P=0.03$) and both IgM RF levels ($P=0.03$) and IgM RF positivity (≥ 3.5 IU/ml; $P=0.005$) were significantly higher in the joint complaint group. IgG ACPA and anti-CarP levels and positivity were equal in the groups. Of the persons with joint complaints at the 2-year follow-up, 39.8 and 34.9% had reported pain or stiffness at baseline, respectively. There was no difference between the groups regarding positivity for more than one autoantibody.

Development of RA

Ten participants (5.6%) reported having developed RA between baseline and the 2-year follow-up. They all reported positively on the CSQ regarding joint stiffness, and joint, hand and wrist pain; nine reported complaints for >3 months and on both sides of the body, five reported difficulty of making a fist, four had hand and wrist swollen, and three had morning stiffness for >6 weeks. Of these 10 persons, 7 were also seropositive for IgA ACPA and IgM RF, 5 for IgA RF and 2 for anti-CarP at baseline.

TABLE 2 Joint complaints in ACPA-positive participants with CSQ data during follow-up (median 23 months)

N = 178	
Joint complaints, n (%)	
1: Joint stiffness in the morning	48 (26.9)
2: Joint complaints for >3 months	62 (34.8)
3: Joint stiffness in the morning lasting at least 1 h for >6 weeks	18 (10.1)
4: Complaints in the same joints on both sides of your body	45 (25.2)
5: Joint pain	59 (33.1)
6: Pain in the wrist or hand	47 (26.4)
7: Swollen hands or wrist	11 (6.2)
8: Difficult to make a fist	14 (7.9)

CSQ: CTD Screening Questionnaire.

EULAR definition: clinically suspect arthralgia

It was found that 75 persons were defined as clinically suspect arthralgia positive (of which 62 were also in the joint complaints group), 102 as no-clinically suspect arthralgia and 1 had missing data. In Table 4, the

TABLE 3 Baseline information of ACPA-positive participants divided by CSQ data concerning joint complaints ($N = 178$)

Baseline	Joint complaints ($N = 83$)	No joint complaints ($N = 95$)	<i>P</i> -value
Age, years, median (IQR)	49 (43–56)	44 (34–51)	0.005
Gender (female), n (%)	60 (72.2)	52 (54.7)	0.01
BMI, kg/m^2 , median (IQR)	25.1 (23.2–27.70)	24.5 (22.6–26.9)	0.21
Smoking			
Never smoker, n (%)	30 (36.1)	46 (48.4)	0.10
Former smoker, n (%)	37 (44.6)	30 (31.6)	0.08
Current smoker, n (%)	16 (19.3)	19 (20)	0.90
Alcohol (g/day), median (IQR)	3.57 (0–7.14)	3.57 (0–8.57)	0.89
Periodontitis (self-reported), n (%)	14 (16.8)	13 (13.7)	0.56
Joint complaints: pain hands and/or feet, n (%)	33 (39.8)	6 (6.3)	<0.001
Joint complaints: stiffness in hands and/or feet, n (%)	29 (34.9)	8 (8.4)	<0.001
FDR with rheumatism, n (%)	21 (25.3)	13 (13.6)	0.05
ACPA IgG pos, n (%)	83 (100)	95 (100)	NA
ACPA IgG level, median (IQR)	19 (9.0–64.0)	17.0 (8.0–61.0)	0.39
ACPA IgA pos n (%)	47 (56.6)	50 (52.6)	0.61
ACPA IgA level, median (IQR)	1.1 (0.6–2.4)	1.1 (0.6–1.9)	0.61
RF IgA pos, n (%)	21 (25.3)	19 (20.0)	0.42
RF IgA level, median (IQR)	6.7 (2.0–25.5)	3.0 (1.2–17.3)	0.03
RF IgM pos (>3.5), n (%)	42 (50.6)	28 (29.5)	0.005
RF IgM pos (>10.0), n (%)	28 (33.7)	20 (21.1)	0.06
RF IgM level, median (IQR)	4.0 (1.1–17.5)	2.3 (0.6–8.5)	0.03
Anti-CarP pos, n (%)	13 (15.6)	12 (12.6)	0.44
Anti-CarP level, median (IQR)	100 (46–237)	89 (43–287)	0.46

Significant *P* values are shown in bold. CSQ: CTD Screening Questionnaire; IQR: interquartile range; anti-CarP: anti-carbamylated protein antibody; FDR: first-degree relative; pos: positive; NA: not applicable.

characteristics between these groups are compared and, next to the already detected differences in the joint complaints group, the number of never smokers was higher in the no-clinically suspect arthralgia group, and levels of IgG ACPA, IgA ACPA and IgM RF were significantly higher in clinically suspect arthralgia group. In Fig. 1, the distribution of arthritis autoantibodies in clinically suspect arthralgia and no-clinically suspect arthralgia groups is shown.

In the clinically suspect arthralgia group, significantly more persons were seropositive for the combination of IgG ACPA, IgM RF and anti-CarP, and more persons were seropositive for three or more autoantibodies. Autoantibody positivity and levels were investigated in the clinically suspect arthralgia group in persons with an FDR only ($n = 21$) and in those with FDR and/or joint complaints ($n = 53$). Of note, FDR only persons may have responded positively to the joint complaint questions that were not used in our clinically suspect arthralgia definition. FDR only persons tended to have lower positivity for and lower levels of IgM RF and IgA RF; however, these were not statistically significant. No further differences were found between these groups (data not shown).

In multivariate logistic regression correcting for age, gender and never smoking, joint complaints [pain hands and/or feet (odds ratio: 3.88, 95% CI: 1.74–8.65); and stiffness in hands and/or feet (odds ratio: 3.72, 95% CI: 1.63–8.50)] and three or more autoantibodies positive (odds ratio: 2.63, 95% CI: 1.04–6.65)

remained significantly associated with clinically suspect arthralgia. Furthermore, there was a trend for ACPA IgA ($P = 0.05$). ACPA IgG level, RF IgM positivity and RF IgM level did not remain significantly different between clinically suspect arthralgia and no-clinically suspect arthralgia.

Evaluation of anti-*P. gingivalis* antibodies, joint complaints and periodontitis

Self-reported periodontitis and levels of anti-*P. gingivalis* at baseline were not different between persons that completed the CSQ or not, nor between the persons with and without joint complaints, or between clinically suspect arthralgia nor no-clinically suspect arthralgia groups.

Of note, anti-*P. gingivalis* levels were significantly correlated to IgM RF ($\rho = 0.30$, $P = 0.006$) and IgA RF levels ($\rho = 0.30$, $P = 0.007$) in the joint complaints group, and there was a trend ($\rho = 0.21$, $P = 0.06$) for a positive correlation with IgG ACPA. In the clinically suspect arthralgia group, anti-*P. gingivalis* levels were significantly correlated to IgG ACPA ($\rho = 0.28$, $P = 0.014$), IgA ACPA ($\rho = 0.23$, $P = 0.05$) and IgM RF ($\rho = 0.36$, $P = 0.001$), and highly significantly to IgA RF levels ($\rho = 0.36$, $P = 0.0006$). There were no significant correlations with anti-*P. gingivalis* levels in the no joint complaints and no-clinically suspect arthralgia groups.

When looking at the association between anti-*P. gingivalis* and specific periodontitis-related questions, significantly more individuals had visible teeth roots in the

TABLE 4 Baseline information of ACPA-positive participants divided by clinically suspect arthralgia characteristics (N = 177)

Baseline	Clinically suspect arthralgia (N = 75)	No-clinically suspect arthralgia (N = 102)	P-value
Age, years, median (IQR)	49 (42–55)	45 (34–51)	0.005
Gender (female), n (%)	53 (70.7)	58 (56.9)	0.06
BMI, kg/m ² , median (IQR)	25.1 (23.3–27.4)	24.6 (22.4–27.3)	0.31
Smoking			
Never smoker, n (%)	22 (29.3)	53 (52.0)	0.003
Former smoker, n (%)	36 (48.0)	31 (29.4)	0.02
Current smoker, n (%)	17 (22.7)	18 (17.6)	0.41
Alcohol (g/day), median (IQR)	4.1 (0.0–9.3)	3.1 (0.0–7.1)	0.50
Periodontitis (self-reported), n (%)	13 (17.3)	14 (13.7)	0.51
Joint complaints: pain hands and/or feet, n (%)	27 (36.0)	12 (11.8)	<0.001
Joint complaints: stiffness in hands and/or feet, n (%)	26 (34.7)	11 (10.8)	<0.001
ACPA IgG pos, n (%)	75 (100)	102 (100)	NA
ACPA IgG level, median (IQR)	22 (10–122)	16.5 (8–44)	0.04
ACPA IgA pos, n (%)	44 (58.7)	50 (49.0)	0.20
ACPA IgA level, median (IQR)	1.39 (0.61–3.93)	1.00 (0.52–1.71)	0.02
RF IgA pos, n (%)	22 (29.3)	18 (17.6)	0.07
RF IgA level, median (IQR)	5.1 (1.6–46.3)	3.4 (1.4–15.1)	0.13
RF IgM pos (>3.5), n (%)	37 (49.3)	32 (31.4)	0.02
RF IgM pos (>10.0), n (%)	26 (38.7)	22 (21.5)	0.05
RF IgM level, median (IQR)	3.1 (1.2–17.5)	2.1 (0.6–7.2)	0.02
Anti-CarP pos, n (%)	13 (17.3)	12 (11.7)	0.06
Anti-CarP level, median (IQR)	110 (53–258)	88 (37–166)	0.30
Positivity for number of arthritis autoantibodies, n (%)			
Only IgG ACPA pos	26 (34.7)	39 (38.2)	0.63
1 extra autoAb pos	15 (20.0)	34 (33.3)	0.05
2 extra autoAb pos	10 (13.3)	13 (12.7)	0.91
3 or 4 extra autoAb pos	24 (34.6)	16 (15.7)	0.01
IgG ACPA + IgM RF + anti-CarP	12 (16)	6 (5.9)	0.03

Significant P values are shown in bold. QR: interquartile range; anti-CarP: anti-carbamylated protein antibody; pos: positive; autoAb: autoantibody; NA: not applicable.

joint complaints group (30.1 vs 14.7%) and in the clinically suspect arthralgia group (29.3 vs 14.7%). The number of persons with loose teeth and bleeding gums were not significantly different between clinically suspect arthralgia and no-clinically suspect arthralgia groups (Table 5).

Discussion

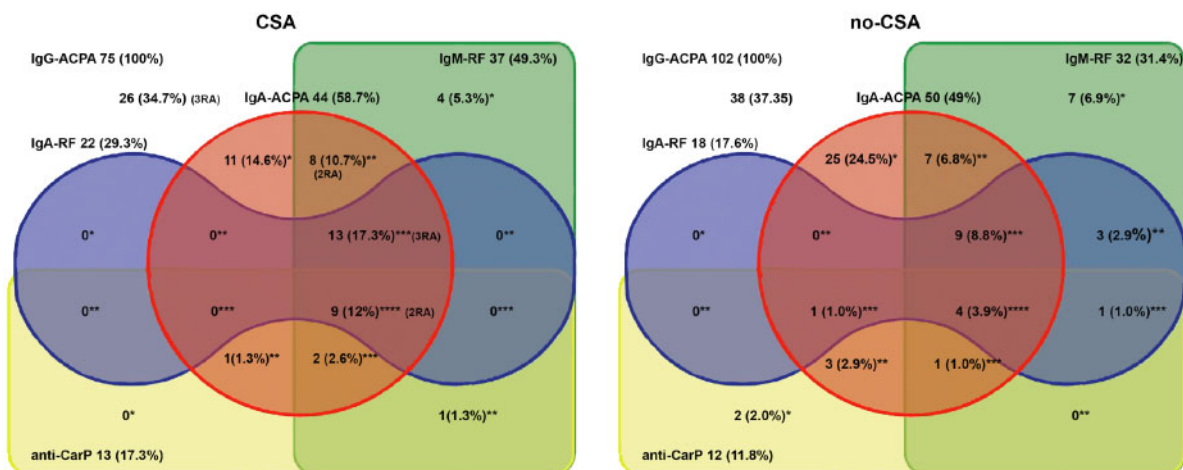
ACPA-positive individuals without defined or self-reported RA but with clinically suspect arthralgia have higher levels of multiple arthritis autoantibodies than ACPA-positive individuals without arthralgia. In the clinically suspect arthralgia group levels of anti-*P. gingivalis* were also correlated to other arthritis autoantibodies, but not in the no-clinically suspect arthralgia group.

It is acknowledged that early recognition of RA is important to prevent joint destruction and various comorbidities [22]. Development to RA is a multistep process with different known phases, as mentioned previously [3]. At-risk patients often present with arthralgia, and in

2017 a EULAR study group defined the most important characteristics describing clinically suspect arthralgia at risk for RA [14]. Since the current study is part of a large longitudinal cohort study that started in 2007–13, with first inclusion and in 2012–16 with the follow up CSQ [16], the exact questions as formulated by the EULAR taskforce are not included in the questionnaires used in Lifelines. We chose the questions from the CSQ that, in our opinion, overlapped with the EULAR taskforce characteristics, and used these in the present study.

To investigate systemic autoimmunity in RA, an expansion of the autoantibody repertoire was chosen, including IgA ACPA, IgM and IgA RF, and anti-CarP. Presence of multiple autoantibodies have been previously described in pre-symptomatic individuals. Brink *et al.* [23] showed seropositivity for IgG ACPA (30%), IgM RF (26%) and IgA RF (25%) in a median pre-dating time of 6.2 years before onset of symptoms. In a previous study of the same group, it was shown that IgA RF occurred at a higher frequency and that it was a good predictor in the early pre-symptomatic period [24]. Kelmenson *et al.* [25] recently showed that in pre-RA individuals, occurrence of

Fig. 1 Distribution of numbers of seropositivity (and percentages) for autoantibodies in clinically suspect arthralgia and no-clinically suspect arthralgia persons



Distribution of numbers of seropositivity (and percentages) for IgA ACPA, IgM RF, IgA RF and anti-CarP in IgG ACPA-positive individuals without RA. Persons are divided into clinically suspect arthralgia and no-clinically suspect arthralgia, based on data from follow-up questionnaires. Persons who developed RA during follow-up and their autoantibody profile are placed at the appropriate place. anti-CarP: anti-carbamylated protein antibody; * seropositive for one extra (besides IgG ACPA) autoantibody; ** seropositive for two extra autoantibodies; *** seropositive for three extra autoantibodies; **** seropositive for four extra autoantibodies.

TABLE 5 Periodontal complaints and anti-*P. gingivalis* levels in ACPA-positive participants divided by clinically suspect arthralgia characteristics

	N = 177	Clinically suspect arthralgia (N = 75)	No-clinically suspect arthralgia (N = 102)	P-value
Signs of periodontitis				
Roots of teeth visible, n (%)	39 (22.0)	22 (29.3)	17 (16.7)	0.05
Loose teeth, n (%)	7 (3.9)	2 (2.6)	5 (4.7)	0.45
Easily bleeding gum, n (%)	23 (13.0)	9 (12.0)	14 (13.7)	0.74
Level of anti- <i>P. gingivalis</i> (U/ml), median (IQR)	1.97 (1.05–3.77)	2.12 (1.05–3.83)	1.92 (1.04–3.77)	0.99

Significant P values are shown in bold. QR: interquartile range.

autoantibodies before diagnosis was as follows: IgG ACPA 17.9 years, IgA RF 14.2 years, IgM RF 7.2 years, IgA ACPA 6.2 years, and IgM ACPA and IgA RF both at 5.0 years. Prevalence of IgA autoantibodies is not surprising, since a dominance of IgA-positive plasmablasts was found in a cohort of autoantibody-positive at-risk persons, suggesting that a subset of RA-related autoantibodies may arise from mucosal immune responses [26]. Recently, Holers *et al.* [27] proposed the so-called mucosal-origin hypothesis, suggesting that local production of IgA autoantibodies at mucosal sites may systemically spread due to inflammatory processes, and are then serologically detectable. Interestingly, we recently showed that in healthy persons with periodontitis, local IgA ACPA production was found in gingival crevicular fluid, indicating that periodontitis may play a role in local ACPA production and onset of arthritis [28].

Antibodies recognizing carbamylated (homocitrulline-containing) antigens have been described for the first time in sera of >45% of RA patients, and in 16% of sera of ACPA-negative patients [8]. Moreover, it was suggested that the presence of anti-CarP antibodies was predictive for a more severe clinical course in ACPA-negative RA patients. Recently, in a large cohort of undifferentiated arthritis the additive value of measuring anti-CarP for predicting RA development was determined [29]. It was found that anti-CarP antibodies had no additional value when RA was defined according to the 2010 criteria, but anti-CarP may be useful for prediction in ACPA-negative and RF-negative patients. In our study, anti-CarP was not different between persons with and without joint complaints, but there was a significant difference between clinically suspect arthralgia and no-clinically suspect arthralgia groups. As described in the study by ten Brinck *et al.* [13],

positivity for IgM RF was of greater influence than measuring additional anti-CarP levels.

Porphyromonas gingivalis is hypothesized to play a role in RA risk by inducing the production of ACPA, either by molecular mimicry or epitope spreading [30]. A recent meta-analysis determining the relation between anti-*P. gingivalis* and ACPA showed not only that anti-*P. gingivalis* levels were higher in RA compared with healthy controls, but also that anti-*P. gingivalis* was positively correlated to ACPA [31]. In the clinically suspect arthralgia patients we also found a positive correlation with both ACPA isotypes as well as with both RF isotypes, while this was not the case for the no-clinically suspect arthralgia group. Our current study shows that anti-*P. gingivalis* levels did not correlate with self-reported periodontitis or with specific periodontitis-related questions. Previous studies showed higher levels of anti-*P. gingivalis* in periodontitis patients compared with no periodontitis patients [32–34], but anti-*P. gingivalis* levels cannot be regarded as a measure for periodontal disease [35]. Whether anti-*P. gingivalis* levels can be regarded as predictive for development of RA is questionable as well. In a cohort of arthralgia patients who were seropositive for IgM RF and/or ACPA, anti-*P. gingivalis* levels at baseline were not different in patients who did or did not develop RA during 12 months follow-up [21]. However, a year may be too short to study RA development in seropositive arthralgia patients. The follow-up time in our present study may also not be long enough. Development to RA may require more time, since Nielen *et al.* [6] showed that autoantibodies may be present up to 14 years before diagnosis.

In our analysis, we found an important contribution of smoking to clinically suspect arthralgia, meaning that persons who were previous or current smokers had a significantly higher risk. In the past years many studies, reviewed by Sparks and Karlson [36], investigating cigarette smoking and RA risk, as well as transitions between phases of preclinical RA, were helpful in establishing the lung as a potential initiating site in the pathogenesis of seropositive RA. In our study, we saw a 2.6 higher risk in clinically suspect arthralgia in persons that were previous or current smokers.

So, in conclusion, measuring multiple arthritis autoantibodies in combination with clinical characteristics identifying clinically suspect arthralgia may help in the early identification of RA development. Levels of anti-*P. gingivalis* are not related to self-reported periodontitis or clinically suspect arthralgia, but are correlated to arthritis autoantibodies in clinically suspect arthralgia.

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