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BRIEF REPORT

The prevalence of anticitrullinated protein antibodies increases with age in healthy individuals at risk for rheumatoid arthritis

D Alpizar-Rodriguez[1] · Laure Brulhart[1] · Ruediger B. Mueller[2] · Burkhard Möller[3] · Jean Dudler[4] · Adrian Ciurea[5] · Ulrich A. Walker[6] · Ines Von Mühlenen[6] · Diego Kyburz[6] · Pascal Zufferey[7] · Michael Mahler[8] · Sylvette Bas[1] · Danielle Gascon[1] · Céline Lamacchia[1] · Pascale Roux-Lombard[9] · Kim Lauper[1] · Michael J. Nissen[1] · Delphine S. Courvoisier[1] · Cem Gabay[1] · Axel Finckh[1]

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Abstract Transition from genetic risk to the development of systemic autoimmunity associated with rheumatoid arthritis (RA) is considered a key step for the development of RA and often referred to as the immune onset of the disease. The aim of this study is to identify predictors for the presence of anticitrullinated protein antibodies (ACPA) as a marker of systemic autoimmunity associated with RA in a high-risk population, an ongoing cohort of first-degree relatives of patients with RA. We assessed the presence of ACPA in individuals without clinical evidence of RA. We examined characteristics associated with ACPA positivity using general estimation equations to account for multiple observations per individual. A total of 1159 serum samples from 1025 subjects were analyzed, 69 samples (6%) were ACPA-positive, and 227 (20%) positive for rheumatoid factor. Participants had a median age of 45 years (interquartile range (IQR): 33–55) at baseline and 76% were women. Overall, ACPA positivity increased with age ($p < 0.001$). Among women, ACPA positivity was particularly associated with the age group 45 to 55 years ($p = 0.003$), but not among men ($p = 0.7$). In multivariable adjusted analyses, age older than 45, female sex and tobacco smoking were independently associated with ACPA positivity. In our cohort, the presence of ACPA was associated with older age and peaked in women around age 45 to 55 years, the perimenopausal period, suggesting that the development of ACPA may be favored by the decline in ovarian function.

Keywords Autoantibodies · Epidemiology · Observational studies · Rheumatoid arthritis

✉ D Alpizar-Rodriguez
Deshire.AlpizarRodriguez@hcuge.ch

[1] Division of Rheumatology, Department of Internal Medicine Specialties, University Hospitals of Geneva, 26, Ave Beau-Sejour, CH 1211 Geneva, Switzerland

[2] Division of Rheumatology, Immunology and Rehabilitation, Kantonsspital St. Gallen, St. Gallen, Switzerland

[3] Inselspital, Bern, Switzerland

[4] HFR, Fribourg, Switzerland

[5] USZ, Zurich, Switzerland

[6] USB, Basel, Switzerland

[7] CHUV, Lausanne, Switzerland

[8] Inova Diagnostics, San Diego, CA, USA

[9] Division of Immunology and Allergy, Department of Internal Medicine Specialties, University Hospitals and Faculty of Medicine of Geneva, Geneva, Switzerland

Introduction

The etiopathogenesis of rheumatoid arthritis (RA) is only partially understood and is thought to result from a multi-step process whereby environmental factors induce a pathological activation of the immune system in genetically susceptible individuals, leading to systemic autoimmunity and subsequently to the clinical onset of the disease [1]. Specific pre-clinical phases of RA development have been proposed, including a stage of "systemic autoimmunity associated with RA," considered as the immunological onset of the disease [2]. It has been postulated that the risk factors driving the transition from one pre-clinical phase to another may differ.

Systemic autoimmunity is characterized by the presence of autoantibodies, such as rheumatoid factor (RF) and anticitrullinated protein autoantibodies (ACPA). Studies have shown that the presence of RF and ACPA precede the onset of

RA by several years [3, 4]. Both ACPA and RF are components of RA classification criteria and define a more severe phenotype, characterized by a more rapid structural joint damage and functional impairment [4, 5]. Several ACPA and RF isotypes are more prevalent in the first-degree relatives of patients with RA (RA-FDRs) [6]. Among blood donors with a positive ACPA test, the risk of developing RA within 5 years was 5% in the general population, but 69% among individuals who had more than two first-degree relatives with RA [7]. Genetic and environmental factors interact to favor the development of ACPA in RA [3, 4]; however, the risk factors for the development of ACPA in a healthy population have not been well-established.

The aim of this study is to identify predictors for the presence of ACPA, as a marker of systemic autoimmunity associated with RA, in individuals without evidence of RA, but genetically at an increased risk.

Methods

Study design

This is an ongoing Swiss multicenter cohort study (SCREEN-RA) of RA-FDRs [8], comprising subjects without a diagnosis of RA at inclusion. Study participants are enrolled in Geneva, Lausanne, Zurich, Bern, St. Gallen, Basel, and Fribourg. RA-FDRs answer a questionnaire about potential environmental risk factors and are examined by a rheumatologist or a specialized study nurse to rule out the presence of RA, other autoimmune conditions, possible tender or swollen joints. Serum samples are collected for genetic testing and autoantibodies (RF, ACPA) assessment. RA-FDRs are followed annually to assess for the development of signs and symptoms of arthritis. To examine the risk factors for the development of ACPA before clinical apparent RA occurred, we further restricted the analysis to individuals who did not develop RA during follow-up nor had any symptoms suggestive of "possible RA," based on the connective tissue disease screening questionnaire (CSQ) [9]. The protocol was approved by the local ethics committee and all participants signed an informed consent before enrolment.

Variables assessed in the analysis were demographic data, such as age, sex, and various putative environmental factors, such as ever tobacco smoking, heavy smoking (smoking >10 pack-years), current alcohol consumption and frequency of consumption (occasionally, every week, every day, or more than one glass by day), and overweight/obese status using the WHO definition (BMI $\geq$ 25 kg/m^2). Age was categorized based in quartiles (<35, 35–45, 45–55, $\geq$55). Other variables examined were joint symptoms (self-reported and on examination), more than one FDR with RA, the presence of shared epitope (SE $\geq$ 1 copy), tooth loss, and poor oral health defined by the presence of any of the following variables: bleeding on brushing, gingivitis, loss of bone around teeth, or mobile teeth.

Study outcomes

The primary outcome of the study was systemic autoimmunity associated to RA, defined by ACPA positivity, operationally characterized by a positive result to any of the anti-cyclic citrullinated peptide antibodies tests (anti-CCP 2.0, 3.0, or 3.1). Autoantibodies were measured using standard, commercially available ELISA kits anti-CCP 2 (CCPlus® Immunoscan, Eurodiagnostica), anti-CCP 3.1 (QUANTA Lite® CCP3.1 IgG/IgA, Inova Diagnostics), or anti-CCP 3 (QUANTA Lite® CCP3 IgG). A secondary outcome was the presence of RF, using the QUANTA Lite IgM and IgA® ELISAs and QUANTA Flash® IgM, and IgA chemiluminescent immunoassays (research use only, Inova Diagnostics). ACPA and RF positivity was defined by a positive test according to the manufacturers' cut-off values (Anti-CCP2 $\geq$ 25 U/mL, anti-CCP3.1 and 3 $\geq$ 20 U/mL, RF QUANTA Lite $\geq$6 U/mL, and RF QUANTA Flash $\geq$ 10344 RLU for IgM and $\geq$7425 for IgA) [10].

Statistical analysis

We used general estimation equations (GEE) analysis with a log link and robust variance estimates to assess the relative risk (RRs) of ACPA positivity associated with predictors of interest. We analyzed univariable and multivariable associations, adjusting for potential confounders (see variables listed in Tables 1 and 2). We evaluated multiplicative interactions for SE-smoking and age-smoking, as previously published [11]. We further performed sub-analyses by type of ACPA tests (anti-CCP 2.0, 3.0, 3.1) and for RF tests. Finally, in order to explore the level of ACPA over time, we used mixed-effects linear regression for longitudinal data in individuals with at least two sequential ACPA assessments. *P* values less than 0.05 were considered statistically significant. Sporadically missing covariates data were managed using multiple imputations. All analyses were performed with STATA 14.0 (Stata Corp LP, College Station, Tx, USA).

Results

Among the 1099 RA-FDRs available in the SCREEN-RA cohort, 34 did not have any ACPA assessment, 7 developed RA during follow-up, and 39 had symptoms related to possible RA (by CSQ) and were excluded. A total of 1025 RA-FDRs, with a median age at baseline of 45 years (interquartile range (IQR): 33–55), contributing 1159 serum samples were analyzed, of which 69 (6%) were ACPA-positive and 227(20%) were RF-positive.

Table 1 General characteristics of participants by ACPA positivity[a]

Characteristics	ACPA-negative n = 1090 (94%)	ACPA-positive n = 69 (6%)	Univariable analysis RR(95%CI)
Age, median (IQR)	45 (33–55)	52 (47–59)	1.0 (1.0–1.1)
Age groups, *n* (%)			
<35	313 (29)	9 (13)	Ref
35–45	237 (22)	5 (7)	0.7 (0.2–2.4)
45–55	275 (25)	33 (48)	3.8 (1.6–9.2)
≥55	265 (24)	22 (32)	2.7 (1.1–6.9)
Sex (female), *n* (%)	826 (76)	62 (90)	2.7 (1.1–6.5)
White European, *n* (%)[b]	1021 (94)	65 (94)	1.1 (0.3–3.6)
Ever smoking, *n* (%)[c]	510 (47)	43 (62)	1.8 (1.0–3.3)
Heavy smoking, *n* (%)	124 (11)	13 (19)	1.7 (0.9–3.4)
Current alcohol consumption, *n* (%)[b]	870 (80)	55 (80)	0.9 (0.5–2.1)
Never	220 (20)	14 (20)	1
Ocasionally/every week	774 (71)	47 (68)	0.9 (0.4–1.9)
Everyday/>1 glass by day	96 (9)	8 (2)	1.2 (0.5–3.2)
Overweight/obese, *n* (%)[c]	364 (33)	24 (35)	1.1 (0.6–2.1)
Lost teeth, *n* (%)[b]			
<5	824 (76)	44 (64)	Ref
5–9	170 (16)	14 (20)	1.5 (0.8–2.8)
>9	96 (9)	11 (16)	2.0 (0.9–4.5)
Poor oral health, *n* (%)	345 (32)	27 (39)	1.4 (0.8–2.4)
Tender joint ≥ 1 examination, *n* (%)	239 (22)	16 (23)	1.1 (0.6–1.9)
Swollen joint ≥ 1 examination, *n* (%)	119 (11)	12 (17)	1.7 (0.9–3.2)
RF, *n* (%)	214 (20)	13 (19)	0.9 (0.5–1.8)
SE (≥1), *n* (%)[b]	566 (52)	41 (59)	1.3 (0.7–2.4)
>1 relative with RA, *n* (%)[b]	172 (16)	13 (19)	1.2 (0.6–2.6)

ACPA anticitrullinated protein antibodies, *RF* rheumatoid factor, *SE* shared epitope

[a] 1159 serum collections from 1025 subjects. Relative risks were calculated by univariable GEE analysis

[b] Sporadically missing in 5–7%

[c] Sporadically missing in 2–3%

In univariable analyses (Table 1), ACPA-positive individuals were significantly older, predominantly female, and more often tobacco smokers. Among women, ACPA positivity was particularly associated with age 45 to 55 years (RR 4.5 (95% confidence interval (CI): 1.7–12.1)), and to a lesser degree with age ≥ 55 years (RR 2.6 (95% CI: 0.9–7.6)), compared to women of less than 35 years. ACPA positivity was strongly associated with the age group 45 to 55 in women ($p = 0.003$), but not in men ($p = 0.7$). We observed a trend for increasing prevalence of RF positivity with age, but without sex difference (Table 2, Fig. 1). Neither the interactions between tobacco smoking (ever) and SE ($p = 0.2$), tobacco smoking and age ($p = 0.1$), neither tobacco smoking (heavy) nor SE ($p = 0.4$) were significant in our cohort.

In the multivariable adjusted model, age older than 45, female sex and tobacco smoking were independently associated with ACPA positivity, irrespective of the specific ACPA test. Being associated in the age group of 45 to 55 years was significantly associated with anti-CCP2 and anti-CCP3.1 positivity and tended to be associated with anti-CCP3 positivity, but did not reach significance (Table 3). In an analysis restricted to subjects with sequential ACPA assessments ($n = 125$), we observed a slow increase of ACPA titers (anti-CCP3) over the years ($p = 0.06$).

Discussion

The present study focused on the identification of predictors for the development of ACPA, as a marker of systemic autoimmunity associated with RA in healthy RA-FDRs. We found a prevalence of 6% of ACPA positivity, in line with the prevalence reported by other RA-FDR cohorts [11, 12]. The presence of ACPA was associated with female sex, age, and tobacco smoking. The association with age was particularly strong in women around the perimenopausal period, with a

Table 2 General characteristics of participants by RF positivity[a]

	RF negative n = 929 (80%)	RF positive n = 227 (20%)	Univariable GEE RR(95%CI)
Age, median (IQR)	46 (34–55)	47 (33–57)	1.0 (0.9–1.0)
Age groups, *n* (%)			
<35	258 (28)	64 (28)	Ref
35–45	202 (22)	39 (17)	0.8 (0.6–1.2)
45–55	249 (27)	57 (25)	0.9 (0.7–1.3)
≥55	220 (24)	67 (29)	1.2 (0.8–1.6)
Gender (female), *n* (%)	710 (76)	175 (77)	1.0 (0.8–1.4)
White European, *n* (%)	873 (94)	209 (92)	0.8 (0.5–1.2)
Smoking ever, *n* (%)	453(49)	98 (43)	0.8 (0.6–1.1)
Alcohol, *n* (%)	339 (36)	76 (34)	0.9 (0.7–1.2)
Overweight/obese, *n* (%)	318 (34)	71 (31)	0.9 (0.7–1.2)
Lost teeth, *n* (%)			
<5	693 (75)	170 (75)	Ref
5–9	155 (17)	29 (13)	0.8 (0.5–1.2)
>9	81 (9)	28 (12)	1.3 (0.9–1.8)
Poor oral health, *n* (%)	289 (31)	82 (36)	1.2 (0.9–1.6)
Tender joint > 1 examination, *n* (%)	197 (21)	59 (26)	1.2 (0.9–1.6)
Swollen joint > 1 examination, *n* (%)	108 (12)	27 (12)	1.0 (0.7–1.5)
SE (≥1), *n* (%)	491 (53)	112 (50)	0.9 (0.7–1.2)
>1 relative with RA, *n* (%)	145 (16)	39 (17)	1.1 (0.8–1.5)

ACPA anticitrullinate protein antibodies, *RF* rheumatoid factor, *SE* shared epitope

[a] 1156 serum collections from 1025 subjects. Relative risks were calculated by univariable GEE analysis

decrease in the prevalence of ACPA in older women, suggesting that the development of ACPA may be facilitated by ovarian aging. Regarding RF, we also observed a trend for an increasing prevalence with age, but did not find a difference by gender or peak in prevalence around the perimenopausal period.

The prevalence of RF positivity increases with age in the general population, irrespective of gender, reaching 25% among individuals older than 85 years [13]. However, the effect of age on ACPA positivity has been less studied. The prevalence of ACPA has been examined in older populations than our cohort, with a median age between 60 to 70 years [14]. In a study in which samples collected from 3116 patients with RA and 15,542 participants with other rheumatic diseases were examined (non-RA patients), ACPA positivity was unrelated to age in both RA and non-RA patients [15]. However, in a Japanese study that included 9804 general population volunteers without any autoimmune disease, age older than 70 years was associated with ACPA positivity, with an odds ratio (OR) of 2.5 (95% confidence interval [95% CI]: 1.5–4.2) [16]. In a prospective cohort of 966 FDRs without RA, the authors found an interaction between smoking, older age (≥50 years), and inflammatory joint signs [11]. To our knowledge, our study is the first to demonstrate the association of ACPA positivity with an increasing age in healthy individuals at risk for rheumatoid arthritis.

We focused on an earlier stage of RA development, namely, autoimmunity associated with RA, defined by ACPA positivity, in a population at a high risk. Interestingly, our findings regarding ACPA positivity are in line with the established difference in age and gender-ratio for RA, with a female to male ratio above four in patients younger than 50 years and below two in patients older than 60 years [17]. In our study, the effect of age on ACPA positivity was strong in women, but not significant in men. Nonetheless, the number of ACPA-positive men was too low to make a definite conclusion about a sex difference. Several studies have demonstrated the presence of increasing levels of different autoantibodies in elderly people, such as antinuclear autoantibodies or rheumatoid factor [13, 18, 19]. The prevalence of antinuclear autoantibodies was 3% in young healthy blood donors and 12% in nonagenarians [19]. This phenomenon has been explained by the increased apoptotic activity related with the aging process and with inmunosenescence [20]. While we also found an increase prevalence of ACPA positivity with age, we did not observe a linear increase, but a peak of prevalence of ACPA positivity around 50 years in women (Fig. 1). The period of age between 45 and 55 years corresponds to the

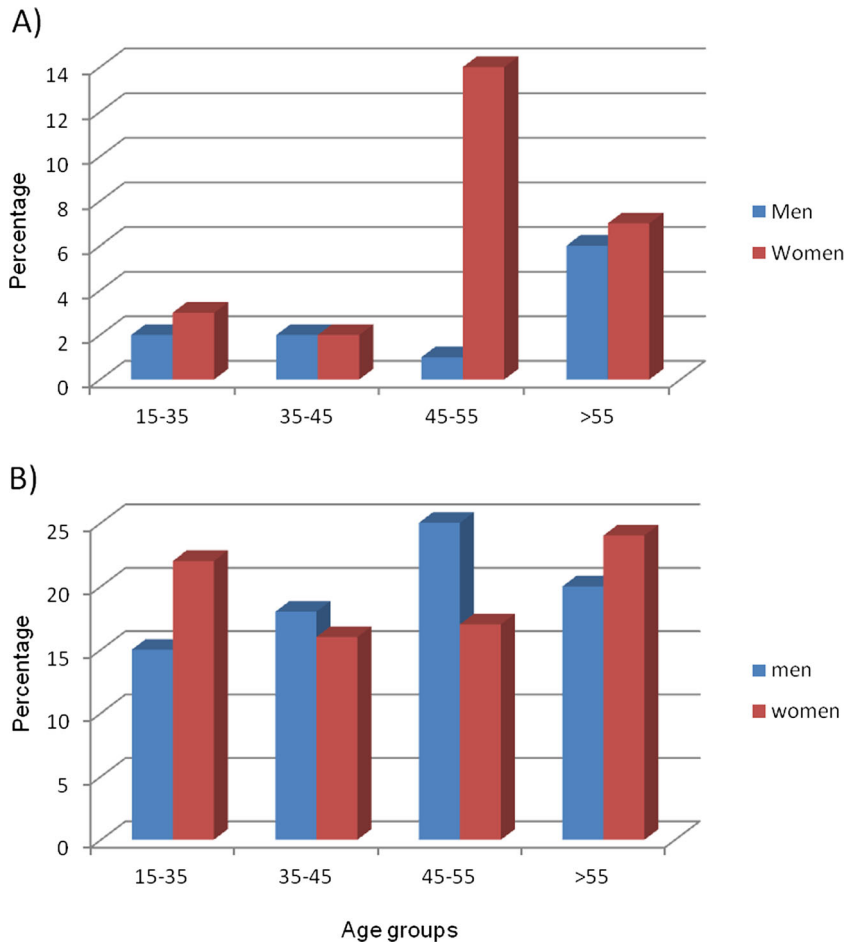

Fig. 1 Percentage of **a** ACPA positivity and **b** RF positivity by sex and age groups

perimenopausal period in women. The rapid decline in ovarian function and in circulating estrogens at menopause is associated with an increase in pro-inflammatory cytokines and earlier age at postmenopause with an increase risk of RA [21, 22]. Alternative hypotheses for this observation could be immunosenescence, changes in diet, in physical activity or in other environmental exposures with aging. The association of tobacco smoking and ACPA positivity, although only significant in univariable analyses, was in line with previous studies on RA development [11, 16].

In sensitivity analyses, we examined three different commercially available anti-CCP tests and found comparable results between the different tests with the overall analysis, suggesting that our findings were not driven by a particular test.

Table 3 Number of serum collections, percentage, RR, and 95% CIs[a] of positivity for ACPA tests

	All ACPA	Anti-CCP2	Anti-CCP3.1	Anti-CCP3
Number	1159	1068	1001	1105
Percentage of positive test, *n* (%)	69 (6)	17 (2)	43 (4)	35 (3)
Age groups				
<35	Ref	Ref	Ref	Ref
35–45	0.7 (0.2–2–2)	2.6 (0.2–27.1)	0.7 (0.1–4.4)	0.5 (0.1–1.9)
45–55	3.4 (1.5–8.1)	9.7 (1.3–76.3)	6.4 (1.9–21.2)	1.6 (0.6–4.3)
≥55	2.4 (0.9–6.0)	4.1 (0.4–37.5)	4.5 (1.3–15.5)	1.1 (0.3–3.7)
Gender (female)	2.7 (1.1–6.5)	1.3 (0.4–4.5)	11.4 (1.6–85.6)	2.5 (0.7–9.0)
Smoking ever	1.6 (0.9–2.9)	1.8 (0.6–5.2)	1.9 (0.9–4.1)	1.8 (0.8–4.0)

ACPA anticitrullinated protein antibodies, *Anti-CCP* anti-cyclic citrullinated peptide antibodies test

[a] Multivariable analyses adjusted for covariates above

To limit potential bias by individuals in pre-clinical phases of RA, we excluded all subjects subsequently diagnosed with RA or with symptoms related to possible RA.

In summary, we demonstrated that in individuals at risk for RA, the presence of ACPA is associated with female sex, tobacco smoking, and older age. In women, ACPA positivity had a strong association around the perimenopausal period, with a decrease in the prevalence of ACPA in older women. This finding suggests that acute decline in ovarian function and estrogen bioavailability may be associated to the development of autoimmunity associated to RA and eventually contribute to the increased risk of the disease in women compared to men.

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Compliance with ethical standards

Disclosures None.

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