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Rheumatoid arthritis autoantibodies and their association with age and sex

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ABSTRACT

Objective. *To examine the association between individual rheumatoid arthritis (RA) autoantibodies, sex and age at RA onset.*

Methods. *Anti-CCP2, IgA-, IgG- and IgM-RF were analysed centrally in baseline sera from 1600 RA patients diagnosed within one year of RA symptom onset. Cut-offs for RF isotypes were determined at the 98th percentile based on RA-free controls, close to the 98.4% anti-CCP2 specificity.*

Results. *Anti-CCP2 was found in 1020 patients (64%), IgA RF in 692 (43%), IgG RF in 529 (33%) and IgM RF in 916 (57%) of the patients. When assessed one by one, anti-CCP2 and IgM RF were both associated with lower age at RA diagnosis. When assessed in one joint model, the association to IgM RF weakened and a strong association between IgA RF and higher age at RA diagnosis appeared. IgA RF and IgG RF associated with male sex, and IgM RF with female sex, with no difference for anti-CCP2.*

When the model was adjusted for sex, the association between IgM RF and age disappeared, whereas the strong associations between IgA RF and high age and between anti-CCP2 and low age at diagnosis remained. Further adjustments for smoking, shared epitope and inclusion year did not change the outcome. Univariate analyses stratified on anti-CCP2 and IgA RF status confirmed the findings.

Conclusion. *Anti-CCP associate with low, and IgA RF with high age at RA onset. RFs and anti-CCP2 display opposing association with sex. These results underscore that studies on RA phenotypes in relation to autoantibodies should accommodate age and sex.*

Introduction

In the 2010 European League against Rheumatism (EULAR)/American College of Rheumatology (ACR) classification criteria for rheumatoid arthritis (RA), the serology domain includes rheumatoid factor (RF) as well as anti-citrullinated peptide antibodies (ACPA), often represented as antibodies against cyclic citrullinated peptide 2 (anti-CCP2).

Early studies showed that patients with RF-positive RA are characterised by lower age of diagnosis as compared to RF-negative patients (2, 3). These studies used isotype-unspecific methods based on agglutination or nephelometry, which primarily, but not exclusively, detect IgM RF. Similarly, the occurrence of ACPA has repeatedly been shown to associate with younger age at RA diagnosis (4, 5). Although there is a strong co-occurrence of RF and ACPA in RA, no studies so far have investigated the association between individual RA autoantibodies and age at RA diagnosis, nor the corresponding association with sex.

Although IgM RF is the most common RF isotype among RA patients of Caucasian ancestry, other RF isotypes appear to be more relevant in other geographical regions. In Asian populations, IgG RF is dominating, or as common as IgM (reviewed in (6)), whereas IgA RF showed the highest sensitivity in a cross-sectional RA cohort from Khartoum, Sudan (7).

The 1987 ACR classification criteria state that RF should be measured "by any method for which the result has been positive in <5% of normal control subjects", and no upper limit of specificity is set (1). No corresponding recommendations exist for ACPA, and the 2010 EULAR/ACR classification criteria do not include any recommendations concerning autoantibody cut-offs. In practice, most laboratories use the cut-offs independently recommended by different manufacturers. These recommendations do, however, differ considerably. In clinical practice, diagnostic laboratories often use higher diagnostic specificity for anti-CCP than for RF, although this has never been officially recommended.

Therefore, and to comprehensively address the question of the occurrence of RA autoantibodies in relation to age at onset and to sex, we examined a large cohort of patients with newly diagnosed RA, in which we centrally measured anti-CCP2 and all three RF isotypes in sera drawn at the time of RA diagnosis, with cut-offs adjusted to the same level in relation to a matched population-based control group.

Patients and methods

Patients

RA patients (n=1600) aged between 18 and 70 years old from the Epidemiological Investigations in Rheumatoid Arthritis (EIRA) incident case-control study were included. All cases (patients with new-onset RA) were diagnosed according to the 1987 ACR classification criteria within one year of first symptoms between 1996 and 2010. Patients included in this study had less than 40 days between the clinical RA diagnosis and inclusion in the EIRA study; blood sampling was performed at inclusion. EIRA controls from the general population were randomly selected from the Swedish population registry, and were individually matched with RA cases for age, sex and residence.

The study was approved by the ethics committee at Karolinska Institutet.

Autoantibody analyses

Anti-CCP2 was measured with the Immunoscan RA ELISA (Eurodiagnostica, Malmö, Sweden), using the 25 U/ml cut-off as suggested by the manufacturer, corresponding to a 98.4% diagnostic specificity among 551 RA-free EIRA controls (9). IgG, IgA and IgM rheumatoid factor (RF) were measured with the EliA immunoassay on a Phadia 2500 instrument (Phadia AB, Uppsala, Sweden) and the cut-offs 17.99 IU/mL, 34.32 µg/mL and 9.96 IU/mL were determined at the 98th percentile among 624 EIRA controls. In alternative calculations, antibody cut-offs were set at the 95.5th percentile.

Statistics

T-tests were used to compare the mean age, and χ^2 tests to compare the proportions of males and females, in patients with and without individual autoantibodies. Linear regression was used to evaluate the association between multiple RA autoantibodies and age at RA diagnosis. We performed models fitted with all RA autoantibodies, and models further adjusted for sex, smoking status (ever/never), year of inclusion and the presence (yes/no) of shared epitope (SE) alleles. *p*-values <0.05 were considered statistically significant.

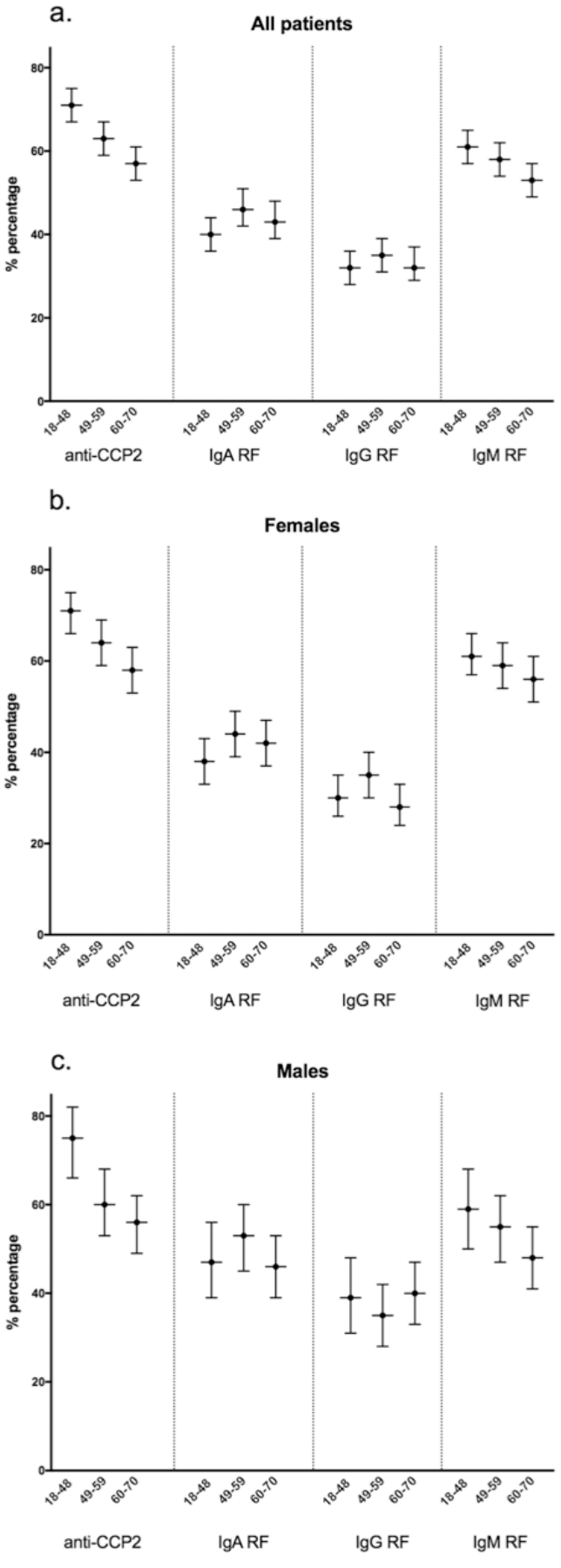

Fig. 1. Autoantibody distribution in relation to and age tertiles among 1600 newly diagnosed RA patients. **a**: shows the data for all patients.

b: the data for females. **c**: the data for males.

Table I. Mean age and gender of 1600 RA patients in relation to the occurrence* of RF isotypes and anti-CCP2. Significant associations are depicted in bold.

	Anti-CCP2 pos/neg	*p*-value	IgA RF pos/neg	*p*-value	IgG RF pos/neg	*p*-value	IgM RF pos/neg	*p*-value
n.	1020/580		692/908		529/1071		916/684	
Univariate models								
Age (mean)	51/54	**<0.0001**	53/52	0.08	52/52	0.86	51/53	**0.0042**
	n (%)		n (%)		n (%)		n (%)	
Females (%)	727/400 (71)/(69)	0.3315	462/665 (67)/(73)	**0.0050**	350/777 (66)/(73)	**0.0089**	665/462 (73)/(68)	**0.0287**
Multivariate models	Std β		Std β		Std β		Std β	
Age model a[1]	0.148	**<0.0001**	-0.154	**<0.0001**	-0.011	0.7170	0.073	**0.0401**
Age model b[2]	0.145	**<0.0001**	-0.139	**<0.0001**	-0.002	0.9378	0.056	0.1128
Age model c[3]	0.146	**<0.0001**	-0.119	**0.0002**	-0.001	0.9688	0.059	0.0918
Age model d[4]	0.147	**<0.0001**	-0.117	**0.0003**	-0.001	0.9720	0.059	0.0950
Age model e[5]	0.152	**<0.0001**	-0.118	**0.0003**	-0.001	0.9612	0.059	0.0924

*Occurrence was determined as above the 98th percentile among population controls for all autoantibodies.
[1]Multivariate analysis including occurrence of anti-CCP2, IgA RF, IgG RF and IgM RF. [2]Model a additionally adjusted for sex. [3]Model b additionally adjusted for never/ever smoking. [4]Model c additionally adjusted for inclusion year. [5]Model d additionally adjusted for shared epitope.

Results

Among the 1600 patients, 1127 (70%) were females. The mean (median) age at RA diagnosis was 54 (57) years among males, and 51 (54) years among women ($p<0.0001$). Anti-CCP2 was found in 1020 (64%) patients, IgA RF in 692 (43%), IgG RF in 529 (33%) and IgM RF among 916 (57%) of the patients. IgM or IgA RF was found in 981 (61%), IgM, IgA or IgG RF in 1023 (64%), and IgM, IgG or IgA RF or anti-CCP2 in 1143 (71%) of the patients. The agreement between the occurrence of the RA autoantibodies varied between poor to good, with the highest kappa value for anti-CCP2 and IgM RF (0.63) and the lowest for anti-CCP2 and IgG RF (0.32; the distribution is shown in Supplementary Fig. S1). In Figure 1, the autoantibody distribution in relation to sex and age (tertiles) is shown.

When investigating one RA autoantibody at a time, anti-CCP2 ($p<0.001$) and IgM RF ($p=0.0042$) were both associated with lower age at RA diagnosis. No such association, rather the opposite, was observed for IgA RF ($p=0.08$), and for IgG RF no association with age was observed ($p=0.86$; Table I).

In models fitted with all RA autoantibodies together, the association between anti-CCP and young age persisted whereas a strong association between IgA RF and higher age at RA diagnosis appeared, and the association between IgM RF and lower age at RA diagnosis weakened (Table I). Variance inflation factors (VIF) for autoantibodies varied between 1.4 and 2.0 and was highest for IgM RF (data not shown). IgA RF and IgG RF associated with male sex and IgM RF with female sex, but no sex-difference was seen for anti-CCP2 (Table I). When the regression was further adjusted for sex, the association between IgM RF and age disappeared, whereas the strong associations between IgA RF and higher age, and between anti-CCP2 and lower age persisted (Table I). Additional adjustment for smoking, age at inclusion in EIRA and occurrence of SE did not change this pattern (Table I). Also, using the 95.5th percentile cut-off for RF isotypes, or for all autoantibodies including anti-CCP2, the same pattern appeared in multiple regression analyses, with appearance of IgA RF association to high age, loss of IgM RF association to low age but with remaining anti-CCP2 association to low age (data not shown).

These results were corroborated in stratified univariate analyses. Whereas anti-CCP2 was associated with young age among RF negative patients after individual stratification for all individual RF isotypes, IgM RF showed no age dependency after stratification for anti-CCP2. IgA RF was associated with high age among anti-CCP2 positive patients (Table II).

Table II. Occurrence of individual RF isotypes in relation to age at RA diagnosis among 1600 RA patients, overall and in subsets of patients defined by the presence or absence of other RA autoantibodies. Significant differences are depicted in bold.

RA autoantibody	Patient subset	Mean age (n) antibody negative patients	Mean age (n) antibody positive patients	*p*-value
IgA RF	All patients	51.5	52.6	0.0781
IgA RF	Anti-CCP2 negative	53.9 (516)	54.1 (64)	0.8546
IgA RF	Anti-CCP2 positive	48.4 (392)	52.4 (628)	**<0.0001**
IgG RF	All patients	52.0	51.9	0.8641
IgG RF	Anti-CCP2 negative patients	54.0 (529)	52.5 (51)	0.4901
IgG RF	Anti-CCP2 positive patients	50.0 (542)	51.8 (478)	**0.0263**
IgM RF	All patients	53.0	51.2	**0.0042**
IgM RF	Anti-CCP2 negative patients	54.0 (489)	53.1 (91)	0.5313
IgM RF	Anti-CCP2 positive patients	50.5 (195)	51.0 (825)	0.6031
Anti-CCP2	All patients	53.9	50.9	**<0.0001**
Anti-CCP2	IgA RF negative patients	53.9 (516)	48.4 (392)	**<0.0001**
Anti-CCP2	IgA RF positive patients	54.1 (64)	52.4 (628)	0.2659
Anti-CCP2	IgG RF negative patients	54.0 (529)	50.0 (542)	**<0.0001**
Anti-CCP2	IgG RF positive patients	52.5 (51)	51.8 (478)	0.3050
Anti-CCP2	IgM RF negative patients	54.0 (489)	50.4 (195)	**0.0009**
Anti-CCP2	IgM RF positive patients	53.1 (91)	51.0 (825)	0.0692

Discussion

In our study, we confirm the previously described association between anti-CCP2 and younger age at RA diagnosis but also demonstrate that the association between IgM RF and age is lost in multivariate analysis. Instead, a strong opposite association between occurrence of IgA RF and higher age at diagnosis appears.

These data imply that the previously reported association between RF and young age (2, 3) is secondary, and that the primary association is between ACPA and young age at diagnosis. The strong opposite association between IgA RF and higher age at RA diagnosis is a new finding. Whereas univariate analyses indicated a mere one-year difference in age, the difference increased to a statistically significant four years difference in multivariate analysis including all autoantibodies, and in univariate analysis stratified for anti-CCP2. This difference remained after adjustment for sex, smoking and shared epitope, when the previously described association between IgM RF and younger age was lost. The association between IgA RF and higher age at diagnosis seems to be restricted to RA. Whereas IgA RF is specifically increased in primary Sjögren's syndrome compared to in RA patients with keratoconjunctivitis sicca investigated in parallel (10), occurrence of IgA RF is associated with more than ten years earlier age at first visit in patients with primary Sjögren's syndrome (11).

A number of previous studies have reported an association between occurrence of IgA RF and the degree of radiological destruction in RA (12, 13). Other studies have shown that patients with RA onset at higher age present with more erosions (14, 15), however IgA RF was not investigated in these studies. It is intriguing to hypothesise that these elderly patients might show more radiological destructions due to occurrence of IgA RF in patients diagnosed at higher age, as we have shown in this report.

The associations observed in this study, were independent on how cut-offs were determined. In the primary calculations all autoantibody cut-offs were set at 98% specificity, but the same results were obtained using 95.5% specificity for all antibodies, or 95.5% for RF isotypes and 98% for anti-CCP2, which is close to the common practice in many clinical laboratories. All cut-off settings are in agreement with the ACR classification criteria (1) and yielded the same results with opposite age associations for anti-CCP2 and IgA RF.

In conclusion, we demonstrate that the occurrence of individual RA-associated autoantibodies at the time of RA diagnosis are differently associated with age at diagnosis and sex. Therefore, future studies evaluating the impact of individual autoantibodies in RA should adjust for or otherwise accommodate age and sex. Such adjustments might be even more important in the future due to migration, as different autoantibody isotypes predominate among RA patients of different ethnicities (6, 7).

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Competing interests

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The other authors have declared no competing interests.

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