Rheumatoid factor and anti-citrullinated peptide antibodies in the general population: hepatitis B and C virus association and 15-year-risk of rheumatoid arthritis

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Abstract

Objective

The study aimed to determine the prevalence of rheumatoid factor (RF) and anti-citrullinated peptides (ACPA), to estimate the association with hepatitis B (HBV) or C (HCV) virus infections and the 15-year risk of developing RA in a large cohort from a Northern Italian region.

Methods

In 1998, 15,907 subjects between the ages of 18 and 75 were randomly selected 1:4 for HBV and HCV testing; more recently, we tested a subgroup of sera for RF (n=2196) and ACPA (n=2525). Administrative databases were searched after 15 years for incident RA diagnoses occurring between 1998 and 2013.

Results

RF was positive in 8.1% of cases with 10% of RF-positive subjects having HBsAg (p=0.004) and 9% anti-HCV. ACPA were detected in 4.8% of subjects with 5% of the ACPA-positive subjects having HBsAg and 5.9% anti-HCV. Older subjects had higher positivity rates for both RF and ACPA. HBsAg and anti-HCV were detected in 5.5% and 4.3% of sera, respectively. Over 15 years, 10 RA cases were recorded (9 women, median age at diagnosis 52 years) with RF previously positive in 2/10 and ACPA in 5/10 cases. RF and ACPA were associated with relative risks for developing RA of 5.7 (adjusted for HBsAg status; 95% CI 1.2–26.3) and 13.2 (95% CI 3.8–46.3), respectively.

Conclusion

Our data in a large cohort from an unselected general population confirm a higher risk of RA development associated with ACPA compared to RF. HBV exposure correlates with RF but not with ACPA positivity.

Key words

rheumatoid arthritis, rheumatoid factor, anti-citrullinated peptide antibodies, HBV, HCV

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Introduction

Rheumatoid arthritis (RA) is a female-predominant autoimmune disease, primarily characterised by a chronic polyarthritis, affecting 0.5–1% of the adult population (1). RA is associated with autoantibodies in the serum and synovial fluid of patients, particularly rheumatoid factors (RF) and anti-cyclic citrullinated peptide antibodies (ACPA) (2), with different sensitivity and specificity profiles for RA development. Serum autoantibodies may be detectable several years before the onset of the disease (3) but are also found in healthy subjects or in association with infections, particularly, viral hepatitis (4), and in the elderly population (5). Healthy subjects who have high-titre RF have an increased risk of RA, especially if multiple isotypes are present (6), but the majority of asymptomatic subjects with RF will never develop RA (7). On the other hand, ACPA are highly specific for RA, having a sensitivity for RA similar to RF (70%) but a higher specificity (>95%) (8, 9). The specificity for RA is maximum if RF and ACPA are both present (10).

Three major issues remain not fully understood, *i.e.* the prevalence of RF and ACPA in the general population, the autoantibody association with hepatitis viruses, and the role in predicting long term risk of RA, as most data are retrospectively derived from selected populations such as blood donors or employees (11). We herein took advantage of a 1998 study in a Northern Italian area (5), characterised by high rates of hepatitis B (HBV) and C virus (HCV) infections, to address these three questions.

Materials and methods

Subjects and study design

The general population of a Northern Italian area in the Lombardy region was screened in 1998 to study the prevalence of HBV and HCV, as previously described (5). Four cities (Bonate Sotto, Ponte San Pietro, Presezzo, Terno d'Isola) including 15907 subjects with ages between 18 and 75 were chosen for the study and citizens were randomly selected 1:4 for participation (n=3977). For the purpose of this study, in 2013 we investigated 2525 sera for ACPA and 2196 for RF without significant differences between groups (Table I). We also performed a retrospective analysis of administrative databases using the ICD-9-codes from the copayment exemptions register (i.e. the Italian legal mechanism that allows subjects with a chronic condition to waive copayments for visits, medications, and blood tests, which are assigned by specialists at the diagnosis of a chronic disease) to identify incident cases of RA between enrollment and December 31st, 2013.

Serological tests

Serum samples were tested in 1998 for the surface antigen of HBV (HBsAg), HBV core antibodies (HBcAb) and anti-HCV antibodies (Chemiluminescent microparticle immune assay, Roche, Basel, Switzerland). Enzyme-linked immunosorbent assay (ELISA, AESKU Diagnostics, Wendelsheim, Germany) was used for RF check as screening test with a cut-off for RF positivity of >16 IU/mL (high titre >50 U/mL, low titre 16-24 U/mL). RF check-positive sera were further tested by ELISA for IgM, IgG, and IgA isotypes (AESKU Diagnostics, Wendelsheim, Germany). In the study of RF isotypes, we first calculated the normal cut-off values testing 20 RA patients, with known RF positivity compared to 20 healthy controls. Reference ranges for RF isotypes were defined by receiver operating characteristic (ROC) curves, with serum samples considered positive if IgG titres were ≥33.6 IU/mL (sensitivity 88.2%, specificity 95.6%), IgM titres ≥18 IU/mL (sensitivity 88.2%, specificity 95.6%), IgA titres ≥18 IU/mL (sensitivity 70.6%, specificity 100%).

ACPA IgG were detected by ELISA (AESKU Diagnostics, Wendelsheim, Germany) and the cut-off was 18 IU/mL (high titre >30 U/mL), as recommended by the manufacturer. Serum samples have also been tested by indirect immunofluorescence (IF) for antinuclear antibodies (ANA) (AESKU Diagnostics, Wendelsheim) in 2525 subjects, as previously reported elsewhere (5).

Statistical analysis

We calculated the cut-off for RF isotypes using ROC curves. ACPA, RF,

Table I. Characteristics of the cohort analysed.

	Subjects tested for RF n=2,196	Subjects tested for ACPA n=2,525
Women, n (%)	1,153 (52.5)	1,363 (54)
Age, median years (IQR)	42 (32-54)	43 (32-54)
Age >60 years, n (%)	301 (13.7)	363 (14.4)
HBsAg-positive, n (%)	117 (5.3)	140 (5.5)
Anti-HBc-positive, n (%)	863 (39.3)	1,002 (39.8)
Anti-HCV-positive, n (%)	83 (3.8)	109 (4.3)
HBV + HCV-positive, n (%)	7 (0.3)	8 (0.3)

RF: rheumatoid factor; n: number; ACPA: anti-cyclic citrullinated peptides; IQR: interquartile range; HB: hepatitis B; Ag: antigen; HCV: hepatitis C virus.

ANA positivity, and HBV/HCV associations were compared between groups using the chi-square test.

The risk ratio (RR) or odds ratio (OR) with 95% confidence intervals (CI) were calculated using logistic regression; when appropriate, the analyses were adjusted for pre-specified confounding variables (*i.e.* sex, age, positivity for hepatitis viruses).

All statistical analyses were performed using Stata 13.1 for Mac (StataCorp LP, College Station, TX, USA), and *p*-values <0.05 were considered statistically significant.

Results

RF and ACPA prevalence

Table II illustrates the prevalence and isotypes of RF, ACPA, and ANA.

RF screening test was positive in 430/2196 sera (19,6%) and confirmed by ELISA in 177/2196 cases (8.1%). High titres of RF were found in 89/177 (50.3%) cases and in 38/42 (90.5%) IgA (*vs.* IgM and/or IgG; *p*=0.03;), and the subjects with RF were cumulatively older than the seronegative group (*p*=0.0047). The ELISA confirmation test was positive in 89/112 (79.5%) subjects with high-titre RF at screening test and in 30/153 (19.6%) with low titres (*p*<0.0001).

Among the 177 RF-positive subjects, 123 (69.5%) had IgM isotype, 117 (66.1%) IgG, and 42 (23.7%) IgA. RF IgM coexisted with IgG isotype in 64 (36.2%) cases; IgM with IgA in 15 (8.5%); IgG with IgA in 25 (14.1%) while all isotypes were positive in 12 (6.8%) subjects. RF IgM and IgG had similar frequencies in both sexes, while IgA was more frequently positive in men (*p*=0.006).

ACPA were detected in 121/2525 (4.8%) subjects (57% women, 20.7% at high titres) and, similarly to RF, ACPA-positive subjects were significantly older than the seronegative population (*p*=0.0006).

RF and ACPA were concomitantly positive in 8/2196 (0.4%) subjects tested for both autoantibodies with 6 subjects having RF IgM + IgG, one RF IgG and one RF IgA.

ANA were positive in 150/2525 (5.9%) subjects, of these 3/150 (8.7%) had ACPA and 3/150 (2%) had RF, with one subject having RF IgM, IgG, and IgA, and 2 subjects RF IgG.

Table II. Prevalence of rheumatoid factor, anti-cyclic citrullinated peptides, and antinuclear antibodies in the study population.

	RF-negative	RF-positive	RF IgM	RF IgG	RF IgA	ACPA-negative	ACPA-positive	ACPA + RF	ACPA + ANA	RF + ANA
n (%)	1,759 (80)	177 (8.1)	123 (5.6)	117 (5.3)	42 (1.9)	2,404	121 (4.8)	8/2,196 (0.4)	13/2,525 (0.5)	3/2,196 (0.14)
Women, n (%)	1,109 (63)	90 (50.8)	68 (55.3)	60 (51.3)	15 (35.7)[¥]	1,110 (46.2)	69 (57)	6 (75)	9 (69.2)	3 (100)
Age, median years (IQR)	43 (32-54)	48 (35-59)*	49 (37-59)*	45 (32-58)	54 (33-56)*	43 (32-54)	47 (38-58)[#]	54 (42-62)	47 (40-52)	60 (40-65)
Age >60 years, n (%)	347 (19.7)	40 (22.6)	28 (22.8)	25 (21.4)	11 (26.2)	337 (14)	26 (21.5)[#]	3 (37.5)	12 (15.4)	2 (66.7)
High titre (%)	-	89 (50.3)	63 (51.2)	57 (48.7)	38 (90.5)[¥]	-	25 (20.7%)	0	3 (23.1)	-
Low titre (%)	-	22 (12.4)	17 (13.8)	13 (11.1)	0	-	-	-	-	-

RF: rheumatoid factor; ANA: antinuclear antibodies; n: number; Ig: immunoglobulin; ACPA: anti-cyclic citrullinated peptides; IQR: interquartile range.
**p*<0.05 *vs.* RF-negative subjects; [¥]*p*<0.05 *vs.* RF IgM/IgG; [#]*p*<0.05 *vs.* ACPA-negative subjects

Table III. Prevalence of hepatitis virus infection according to the rheumatoid factor, anti-cyclic citrullinated peptides, and antinuclear antibodies status in the study population.

	RF- negative	RF- positive	RF IgM	RF IgG	RF IgA	ACPA-negative	ACPA-positive	ACPA + RF	ANA + ACPA	ANA + RF
n (%)	1,759 (80)	177 (8.1)	123 (5.6)	117 (5.3)	42 (1.9)	2,404 (95.2)	121 (4.8)	8/2,196 (0.4)	13 (0.5)	3 (0.14)
HBsAg (%)	88 (5)	18 (10)*	16 (13.2)	12 (10.3)	7 (16.7)	134 (5.6)	6 (5.0)	0	1 (7.7)	0
Anti-HBc (%)	682 (39)	84 (47.7)[¥]	64 (52.9)	50 (42.7)	22 (52.4)	948 (39.4)	54 (44.6)	5 (62.5)	6 (46.2)	1 (33.3)
Anti-HCV (%)	63 (3.6)	16 (9)-	15 (12.4)	12 (10.3)	4 (9.5)	102 (4.2)	7 (5.9)	1 (12.5)	2 (15.4)	1 (33.3)
HBsAg+HCV	6 (0.3)	1 (0.6)	1 (0.8)	1 (0.9)	1 (2.4)	8 (0.3)	0	0	0	0
Anti-HBc+HCV	38 (2.2)	15 (8.5)[#]	14 (11.6)	11 (9.4)	4 (9.5)	72 (3)	5 (4.1)	1 (12.5)	2 (15.4)	1 (33.3)

RF: rheumatoid factor; Ig: immunoglobulin; ACPA: anti-cyclic citrullinated peptides; ANA: antinuclear antibodies; HB: hepatitis B; Ag: antigen; HCV: hepatitis C virus; **p*=0.004 *vs.* RF-negative subjects; [¥]*p*=0.03 *vs.* RF-negative subjects; [#]*p*<0.001 *vs.* RF-negative subjects.

Hepatitis virus prevalence according to autoantibody status

HBsAg was positive in 140/2525 (5.5%) of subjects, anti-HBc in 1002 (39.8%), and anti-HCV in 109 (4.3%). All HBsAg positive subjects also had anti-HBc reactivity and 8 (0.3%) subjects had both anti-HBc and anti-HCV (Table I).

RF-positive subjects had HBsAg in 18/177 cases (10%; *p*=0.004 *vs*. RF-negative; OR adjusted for sex and age 1.92, 95% CI 1.12–3.2), with 16 IgM isotype, 12 IgG, 7 IgA. RF-positive subjects had anti-HBc in 84/177 (47.7%; *p*=0.003 *vs*. RF-negative; OR adjusted for sex and age 1.17, 95% CI 0.8–1.6), 64 (52.9%) IgG, 50 (42.7%) IgM, and 22 (52.4%) IgA. RF-positive subjects had anti-HCV in 16/177 (9%; *p*<0.001 *vs*. RF-negative; OR adjusted for sex and age 1.6, 95% CI 0.9–2.7), 15 (12.4%) IgG, 12 (10.3%) IgM, 4 (9.5%) IgA. Multiple viral reactivity was observed in a minority of RF-positive subjects (Table III, Fig. 1).

ACPA-positive subjects had HBsAg in 6/121 (5%), while they had anti-HBc in 54/121 cases (44.6%). ACPA-positive subjects had anti-HCV in 7/121 (5.9%; Table III).

ACPA and RF were both positive in 1/8 (12.5%) of the subjects with anti-HCV, in no patient with HBsAg and in 5/8 (62.5%) of the subjects with anti-HBc (Table III).

ANA-positive subjects had HBsAg in 6/150 (4%) cases, while they had anti-HBc in 73/150 (48.7%; *p*=0.007 *vs*. ANA-negative; OR adjusted for sex and age 1.11, 95%CI 0.9–1.4). ANA-positive subjects had anti-HCV in 10/150 (6.7%), HBsAg and anti-HCV in 2/150 (1.3%), anti-HBc and anti-HCV in 28/150 (18.7%; data not shown in the tables).

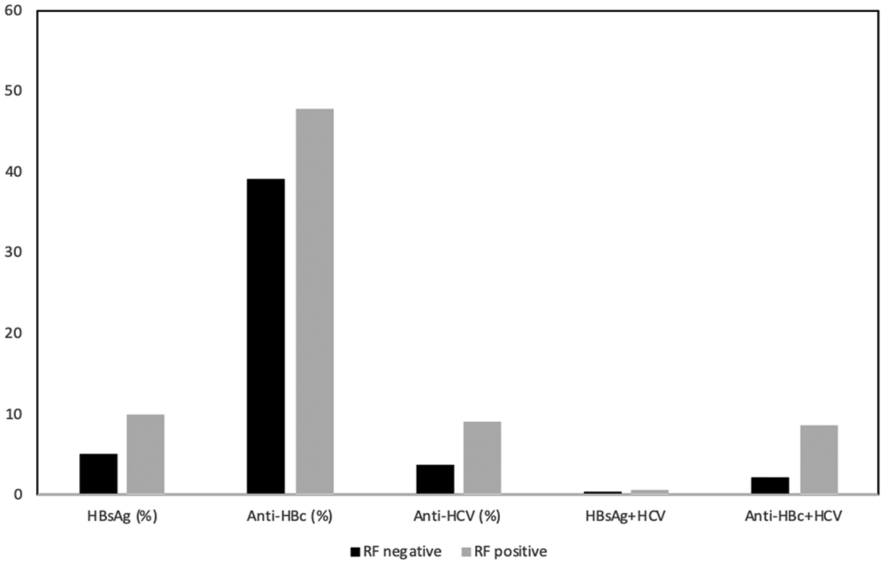

Fig. 1. Prevalence of hepatitis virus infection according to the rheumatoid factor.

Table IV. Number of events and 15-year risk ratios and 95% confidence intervals associated with the autoantibody status.

	Total population	RF-positive n=177	ACPA-positive n=121
Rheumatoid arthritis cases	10	2	4
Risk ratio	–	2.5	13.2
95% CI	–	0.2-27.2 *p*=0.44	3.8-46.3 *p*<0.001

Incidence of rheumatoid arthritis

We identified 10 incident RA cases over a 15-year follow-up (Table IV), 9 of which were women, with a median age at diagnosis of 52 years.

Among RA cases, 2/10 (20%) were previously positive for RF (Table IV), both with IgM isotype, one with low titre RF and coexisting ACPA and one with high titre RF, corresponding to a RR of 2.49 (95%CI 0.23–27.21, *p*=not significant). When taking into account only women, the RR for RA development for RF-positive subjects was 1.77 (0.22–14.06, *p*=not significant). Adjusting the analysis for the significant association between RF and HBsAg, the RR of developing RA if RF-positive was 5.7 (95%CI 1.2–26.3; *p*=0.013). The RF-positive predictive value was 1.13% (95% CI 0.33–3.83%), while the negative predictive value was 90.55% (95% CI 98.4–99.7%).

Among patients with incident RA, 4/10 (40%) were positive for ACPA at enrolment, 3 of which with high titre ACPA and 1 with ANA (for a coexisting Sjögren's syndrome), corresponding to a RR of 13.2 (95%CI 3.8–46.3; *p*<0.001; Table IV). When including only women in the analysis, the RR for RA development among ACPA-positive subjects was 9.38 (2.4-36.7, *p*<0.001), while only one man with ACPA developed RA (1/53, 1.9%; *p*=0.05). The ACPA-positive predictive value was 3.31% (95% CI 1.54–6.94), while the negative predictive value was 99.75% (95% CI 99.6–99.9).

None of the patients developing RA had positive viral markers.

Discussion

Serum autoantibodies are being used in the diagnostic work-up for autoimmune diseases and, particularly RF and ACPA, to discriminate patients with arthralgia suspected of having RA. In the present study, we report the prevalence of serum RF and ACPA and their predictive value for RA development over 15 years of follow-up in a large sample from the general population; furthermore, we investigated the influence of hepatitis viruses on the development of autoantibodies.

We observed that the prevalence of IgM RF (5.6%) was similar to that reported elsewhere (2.8–6.6%) (12-14), but slightly higher considering any RF isotype (8.1%). Our data confirm that IgM and IgG represent the main RF isotypes, while IgA are less frequent. The ACPA prevalence in our cohort

(4.8%) was also similar to previous data (1.5–5%) (12-15). Somehow surprisingly and differently from ANA, the sex ratio was 1 for both RF and ACPA. The frequency of both autoantibodies increased with age, similarly to previous reports (16).

According to the literature, 17–42% of HBV-carrying patients are RF-positive (17), and our findings suggest a significant association between RF and HBsAg, with a 2-fold increase of RF prevalence among HBsAg-positive subjects, but we failed to find an association with RA disease, such as demonstrated by a systematic literature review (4). This is of particular interest since HBV infection may manifest with extra-hepatic features such as arthralgia, and in RA with chronic HBV infection, viral particles have been recently demonstrated in the synovia of affected joints, being associated with a more severe inflammatory infiltrate (18). Of relevance to clinical practice, HBV vaccination in HBV-negative RA patients (19) or the choice of immunosuppressive treatments for RA in patients with chronic HBV infection should be carefully evaluated due to the risk of viral reactivation (20), particularly in endemic areas (21), such as the Northern Italian area that was investigated herein.

Quite surprisingly, we did not find an association between RF and HCV, but in our cohort, we could not distinguish chronic HCV infection and anti-HCV carriers: Moreover, we did not have data on cryoglobulins which are often associated to HCV infection and have a rheumatoid factor activity.

We identified a total of 10 RA cases over a 15-year follow-up, with a female predominance and a peak incidence around 50 years of age, which is consistent with previous reports (22). Our data do not confirm a significant risk of RA development in RF-positive subjects, unless HBV infection is accounted for. Conversely, we found an increased risk of developing RA in ACPA-positive subjects, confirming that ACPA have higher specificity than RF for RA development (23). Of interest, one (10%) RA incident case was seropositive for both ACPA and RF, a status observed in only 0.8% of our population, therefore supporting that the combination of both autoantibodies may represent a major risk factor for RA (23).

We believe that the present study represents a unique setting for the study of prevalence and clinical significance over time of RF and ACPA related to HBV and HCV thanks to several strengths. First, the observational time of our study is one of the longest reported for a population-based longitudinal study in autoimmunity (24). Second, autoantibodies were tested in 2013, thus taking advantage of more modern methods compared to those available in 1998. Third, the administrative health database of the National health system tracks all new diagnoses for a chronic disease, hospital admissions and diagnoses or procedures, thus allowing the determination of occurrence of health-related events over time (25-27). However, we are also aware of the limitations of our approach. Our analysis of administrative databases is limited by the fact that 11% of the general population in 2013 had an exemption due to low income or age over 65 years, and this limits the sensitivity of clinical exemptions for specific diseases such as RA. This limitation is associated with a reduced statistical power and possible misclassification in subjects older than 65 with a low family income. Moreover, we did not have data on anti-HBsAg which would allow us to better define occult HBV infection (28). Finally, all serum samples were only tested at one time point without a subsequent confirmation test on a separate occasion, and no titre variation was studied over time.

In conclusion, our study provides an answer to the previously mentioned major issues, *i.e.* the prevalence of RF and ACPA in the general population, the autoantibody association with hepatitis viruses, and the role in predicting long term risk of RA, and support the view that there is a higher risk of RA development associated with ACPA compared to RF, and that RF positivity is significantly influenced by HBV status but not by HCV.

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