**Arthritis & Rheumatology**
Vol. 72, No. 10, October 2020, pp 1658–1667
DOI 10.1002/art.41381


American College of Rheumatology
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# Anti–Citrullinated Protein Antibody Specificities, Rheumatoid Factor Isotypes, and Incident Cardiovascular Events in Patients With Rheumatoid Arthritis

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**Objective.** To investigate the relationship between anti–citrullinated protein antibodies (ACPAs), specific ACPA subspecificities, rheumatoid factor (RF) isotypes, and incident cardiovascular (CV) events in patients with rheumatoid arthritis (RA).

**Methods.** Serum samples from Swedish patients with new-onset RA (diagnosed within 1 year of symptom onset between 1996 and 2009) were centrally typed for anti–cyclic citrullinated peptide 2 (anti-CCP2) antibodies, 20 ACPA subspecificities, and RF isotypes. Patients were followed up longitudinally in nationwide registers to monitor the occurrence of acute coronary syndrome (ACS), stroke, CV-related death, and major adverse CV events (MACE). The association between each serologic marker and CV outcome, and the impact of adjustment for the Disease Activity Score in 28 joints (DAS28), smoking status, and income at baseline, were assessed using Cox proportional hazards models. In addition, associations of serologic markers with all-cause mortality were explored.

**Results.** In total, 2,814 patients with RA were included in the study. The median follow-up was 13 years, during which the CV end points of ACS, stroke, or CV-related death were reported to occur in 375 patients. Occurrence and/or levels of anti-CCP2 were associated with risk of incident ACS (hazard ratio [HR] 1.46, 95% confidence interval [95% CI] 1.03–2.06), stroke (HR 1.47, 95% CI 1.03–2.10), CV-related death ($P = 0.024$ for association with anti-CCP2 levels), and MACE (HR 1.34, 95% CI 1.06–1.70). Similarly, an association with the number of ACPA subspecificities was observed; however, this could not be attributed to any individual or group of ACPA subspecificities. Presence of IgM-RF was associated with all CV end points except ACS, and IgA-RF was exclusively associated with CV-related death. Adjustment for smoking status, income, and DAS28 scores decreased most of the HRs, whereas IgA-RF remained associated with CV-related death (HR 1.61, 95% CI 1.05–2.48). All of the assessed serologic makers were associated with all-cause mortality.

**Conclusion.** RF isotypes and ACPAs are associated with future CV events in patients with RA. ACPA levels and number of subspecificities seem more important than the occurrence of particular subspecificities, and these associations were not explained by a history of ever smoking.

Supported by the Swedish Research Council, the Swedish Foundation for Strategic Research, Stockholm County Council, the Swedish Heart Lung Foundation, Karolinska Institutet, Nordforsk, and the Rheumatology Research Foundation (a peer-reviewed collaborative research grant from the Foundation for Research in Rheumatology).

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Dr. Serre is the inventor on an international patent held by Biomerieux Cy and licensed to Eurodiagnostica and Axis-Shield for CCP2 assays for which he received part of the royalties paid to Toulouse University and University Hospital. Dr. Askling has current or previous research agreements with AbbVie, AstraZeneca, Bristol Myers Squibb, Eli Lilly, MSD, Pfizer, Roche, Sanofi, Samsung Bioepis, and UCB, mainly in the context of safety monitoring of biologics via the ARTIS/Swedish Biologics Register. No other disclosures relevant to this article were reported.

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Submitted for publication November 1, 2019; accepted in revised form May 21, 2020.

## INTRODUCTION

Patients with rheumatoid arthritis (RA) are at increased risk of cardiovascular (CV) disease (1). We and others have demonstrated a rapid increase in the risk of CV events (2,3), in particular acute coronary syndromes (ACS) (4) but also stroke (5) and CV mortality (6), from no elevation (7) to an already elevated risk (8) following RA diagnosis. Although the underlying risk of CV events in patients with RA, as well as in the general population, has declined during recent years, the risk of CV events in patients diagnosed as having RA remains elevated (4).

The increased risk of CV events in RA cannot be fully attributed to traditional CV risk factors (9), a finding that points to the potential role of risk factors associated with RA (10) or to a shared etiology between RA and CV disease (11). In terms of risk factors for CV events in inception cohorts of patients with RA, we and others have reported an association not only with accrued RA disease activity (12,13) but also with high levels of anti–citrullinated protein antibodies (ACPAs) and risk of ACS (10). Interestingly, ACPAs may also be present in atherosclerotic plaques (14), raising the possibility that, beyond their role as a marker of RA-related inflammation, ACPAs might be linked to CV events through other routes. Similarly, other studies in patients with RA, as well as in subjects from the general population without RA, have linked the presence of rheumatoid factor (RF) to CV risk (15,16).

In these previous assessments, the presence and levels of ACPAs have been defined using the anti–cyclic citrullinated peptide 2 (anti-CCP2) assay. Citrullination is, however, a general phenomenon, and therefore it remains unclear as to whether any particular ACPA subspecificities, or "load" thereof (i.e., the number of subspecificities expressed), are particularly linked to CV events, and whether any association differs across the different types of CV events, such as coronary disease and stroke. Similarly, any independent role of different RF isotypes on CV events in RA has, to our knowledge, not been investigated.

In this study, we therefore aimed to further investigate the association between the presence and levels of anti-CCP2, specific ACPAs and their combinations, and RF isotypes and the risk of ACS, stroke, CV-related death, and a composite end point of major adverse CV events (MACE) in patients with RA who were followed up for these CV outcomes from the time of diagnosis. To this end, we took advantage of prospectively collected data from a large inception cohort of patients with RA, assessed serum samples using a chip-based anti-CCP2 assay (17,18), and utilized linkage of the patients' data to nationwide and population-based registers on incident CV end points to ascertain potential associations between these serologic markers and CV outcomes.

## PATIENTS AND METHODS

**Study design and setting.** The Swedish Epidemiological Investigation of RA (EIRA) study includes patients from the middle and southern regions of Sweden who were newly diagnosed as having RA according to the American College of Rheumatology (ACR) 1987 classification criteria for RA (19); the EIRA study population has been described previously (20,21). At baseline, with informed consent, patients contributed a blood sample and answered questions on a lifestyle questionnaire. From the EIRA cohort, we identified a cohort of patients who were newly diagnosed (within 1 year of symptom onset) as having RA between 1996 and 2009, all of whom fulfilled the ACR 1987 criteria (19). As previously described (22), we linked this RA cohort to the following national Swedish registers: the National Patient Register (NPR), with information on inpatient and specialist care in Sweden, the Cause of Death register, for identification of the underlying cause of death, and the Longitudinal Integrated Database for Health Insurance and Labour Market Studies (LISA), for information regarding income. We also used the Swedish Rheumatology Quality (SRQ) register, from which we retrieved information on clinical presentation at RA diagnosis, and covariates. These linkages used the unique personal identification number as key. Ethics approval for this study was obtained from the Stockholm ethics review board (approval nos. 2015/1844-31/2, 2010/810-32, 2007/889-31/2, and 2005/1387-31).

**Serologic data.** Serum samples were obtained from the EIRA study participants at the time of RA diagnosis. The sera were assessed centrally using a commercial anti-CCP2 assay from Eurodiagnostica. Anti-CCP2 levels were divided into categories of <25 arbitrary units (AU)/ml, 25 to <75 AU/ml, 75 to <1,500 AU/ml, and ≥1,500 AU/ml, in accordance with the manufacturer's instructions (23), with a status of anti-CCP2 negative being defined as a serum level of <25 AU/ml, and anti-CCP2 high-positive as a serum level of ≥75 AU/ml (3 times the reference value as described in ref. 24). Using the manufacturer's suggested anti-CCP2 cutoff level of 25 AU/ml, the diagnostic specificity of the anti-CCP2 assay was 98.4% among 578 EIRA study control subjects.

ACPA subspecificities were centrally tested on a custom-made microarray (Thermo Fisher Scientific) (17). For the present analyses, a total of 20 ACPA subspecificities was included. The cutoff level for the subspecificities was set based on the 98th percentile value among 538 EIRA study general population control subjects (18). IgA-, IgG-, and IgM-RFs were analyzed using an EliA immunoassay on a Phadia 2500 instrument, using cutoff levels set at the 98th percentile value among the RA-free EIRA study controls.

**Follow-up and outcomes.** Using the NPR and Cause of Death registers, we obtained follow-up data on all individuals from the time of their inclusion in the EIRA study until a first event of ACS, CV-related death, stroke, or MACE, defined according to a hospitalization listing with a relevant International Classification of Diseases, Tenth Revision (ICD-10) code (ICD-10 codes I20.0 or I21 for cause of death [any ICD-10 code listed as "I" for CV-related death], ICD-10 codes I60–I64 for stroke, and any of the above ICD-10 codes for MACE). Follow-up was censored at the time of

death, emigration, or the end of the study period (December 31, 2016).

**Statistical analysis.** For the statistical analyses, we included all ACPA subspecificities with a frequency of ≥10% in our sample. We further defined autoantibody "load" as the total number of these subspecificities expressed by an individual, categorized into tertiles based on the distribution in our study population. The Spearman's rank correlation test was used to calculate the correlation between anti-CCP2 levels and number of specific autoantibodies expressed.

For each outcome, we calculated its incidence in the study population, and used Cox proportional hazards models to assess the association of each CV outcome with ACPA/RF status, number of ACPA subspecificities expressed, and anti-CCP2 antibody load. All models were adjusted for age, sex, and calendar period of RA diagnosis. For the analyses of individual subspecificities, we applied Bonferroni's correction using a factor of 76 to adjust for multiple hypothesis testing. We excluded from the analyses of each particular CV end point all individuals who had a history of the outcome of interest prior to the start of follow-up. Where applicable, we did, however, adjust the analyses of each CV outcome for a history of any of the other CV outcomes. Each ACPA subspecificity was analyzed in separate models.

With regard to RF, the isotypes were analyzed in separate models. In addition, we included all RF isotypes jointly in a single model, and ran one model that included all 3 RF isotypes together with anti-CCP2 status.

**Exploratory analysis.** We performed exploratory analyses to investigate the impact of adjustment for each of 3 CV event determinants (smoking status, income [as a proxy for general socioeconomic status, and thus CV events], and RA disease activity at diagnosis) on the associations between RA-specific autoantibodies and the CV end points. With regard to smoking, we collected data on smoking status (never [reference] versus ever smoker) at the time of RA diagnosis as reported by each patient on the EIRA lifestyle questionnaire, and adjusted our main analyses for smoking status. With regard to anti-CCP2 status and RF isotypes, we also performed analyses stratified by smoking status.

For income, we retrieved information from the year before RA diagnosis from the LISA database. Data on income was adjusted for inflation and divided into deciles, with the highest income group used as reference.

For RA disease activity at diagnosis, we retrieved data (when available) from the SRQ registry on each patient's Disease Activity Score in 28 joints (DAS28) at the time of RA diagnosis, and divided the scores into categories of low (DAS28 ≤3.2), medium (3.2 < DAS28 ≤ 5.1), and high (DAS28 >5.1) as reported at the time of the first visit, which was required to be not more than 40 days before or 75 days after the blood sample was obtained.

To contextualize any association with the CV end points, we also investigated the association between the RA antibodies and all-cause mortality. To this end, we retrieved data from the population register on deaths attributed to any cause.

## RESULTS

**Characteristics of the patients.** We identified 2,814 patients with incident RA and with information on the presence or absence of anti-CCP2 antibodies, specific ACPAs, and RF. The median age at the time of RA diagnosis was 51 years (interquartile range [IQR] 18), and median follow-up from the time of RA diagnosis was 13 years (IQR 6.5) (Table 1). Most study participants were born in Sweden (88%) while 9% were born in other European countries and 3% in other parts of the world. Among the 2,814 patients, 1,816 (65%) were anti-CCP2 positive and 1,614 (57%) were IgM-RF positive.

During follow-up, we observed 147 first occurrences of ACS events (incidence 4.3 per 1,000 person-years), 141 first occurrences of stroke events (incidence 4.1 per 1,000 person-years), 87 CV-related deaths (incidence 2.4 per 1,000 person-years), and 313 MACE events (incidence 9.4 per 1,000 person-years).

**ACPA and ACPA subspecificities.** Anti-CCP2 positivity was associated with the risk of incident ACS (hazard ratio [HR] 1.46, 95% confidence interval [95% CI] 1.03–2.06), stroke (HR 1.47, 95% CI 1.03–2.10), and MACE (HR 1.34, 95% CI 1.06–1.70), with a similar effect size for CV-related death although the association was not statistically significant (HR 1.48, 95% CI 0.94–2.31). When categorized by levels of anti-CCP2, there were

**Table 1.** Demographic and clinical characteristics of the Swedish study population (n = 2,814) followed up for incident CV events from RA diagnosis until 2017*

| | ACS | Stroke | CV-related death | MACE |
|---|---|---|---|---|
| No. of patients | 2,814 | 2,814 | 2,814 | 2,814 |
| No. with a history of the CV event in question before RA diagnosis | 32 | 20 | 0 | 50 |
| No. remaining for analysis after exclusion | 2,782 | 2,794 | 2,814 | 2,764 |
| No. (%) women | 2,003 (72.0) | 2,005 (71.8) | 2,015 (71.6) | 1,993 (72.1) |
| Age at RA diagnosis, median (IQR) years | 51.4 (18.0) | 51.4 (18.0) | 51.5 (18.0) | 51.3 (18.0) |
| Follow-up, median (IQR) years | 12.3 (6.5) | 12.4 (6.5) | 12.6 (6.5) | 12.1 (6.5) |
| Incidence of each CV event per 1,000 person-years | 4.3 | 4.1 | 2.5 | 9.4 |

* CV = cardiovascular; RA = rheumatoid arthritis; ACS = acute coronary syndrome; MACE = major adverse cardiovascular events; IQR = interquartile range.

**Table 2.** Association between presence and titers of anti-CCP2 antibodies and incident ACS, stroke, CV-related death, and MACE among 2,814 Swedish patients followed up from RA diagnosis for incident CV events*

| | % of positive patients | ACS† | | Stroke‡ | | CV-related death | | MACE§ | |
|---|---|---|---|---|---|---|---|---|---|
| | | HR (95% CI) | *P* | HR (95% CI) | *P* | HR (95% CI) | *P* | HR (95% CI) | *P* |
| Anti-CCP2 positive | 65 | 1.46 (1.03–2.06) | 0.035 | 1.47 (1.03–2.10) | 0.034 | 1.48 (0.94–2.31) | 0.087 | 1.34 (1.06–1.70) | 0.014 |
| Anti-CCP level | | | | | | | | | |
| Low | 7 | 1.85 (0.96–3.58) | 0.066 | 1.27 (0.60–2.70) | 0.54 | 1.42 (0.55–3.68) | 0.47 | 1.36 (0.83–2.21) | 0.22 |
| High | 43 | 1.37 (0.94–2.00) | 0.1 | 1.42 (0.97–2.09) | 0.073 | 1.23 (0.75–2.03) | 0.41 | 1.29 (1.00–1.66) | 0.053 |
| Extreme | 14 | 1.57 (0.96–2.57) | 0.075 | 1.72 (1.05–2.81) | 0.031 | 2.27 (1.28–4.03) | 0.0053 | 1.52 (1.09–2.12) | 0.014 |
| *P* for trend | – | – | 0.055 | – | 0.020 | – | 0.024 | – | 0.01 |

* Values for the association between presence and titers of anti–cyclic citrullinated peptide 2 (anti-CCP2) antibodies and incident acute coronary syndrome (ACS), stroke, cardiovascular (CV)–related death, and major adverse CV events (MACE) are shown as the hazard ratio (HR) with 95% confidence interval (95% CI). Levels of anti-CCP were defined as follows: low = 25 to <75 AU/ml, high = 75 to <1,500 AU/ml, and extreme = ≥1,500 AU/ml.
† Adjusted for age, sex, age$^2$, and calendar period of rheumatoid arthritis (RA) diagnosis and stroke event before the diagnosis.
‡ Adjusted for age, sex, age$^2$, and calendar period of RA diagnosis and ACS event before the diagnosis.
§ Adjusted for age, sex, age$^2$, and calendar period of RA diagnosis.

statistically significant trends toward increasing incidence of CV events (stroke, CV-related death, and MACE) with increasing anti-CCP2 levels, and this was borderline significant for incidence of ACS (Table 2).

In analyses of specific ACPA subspecificities, 17 autoantibodies were found to have a prevalence of ≥10% in our sample. These included Fil 307–324 (CCP-1), Vim 60–75, Vim 2–17, Fibβ 36–52, Fibα 563–583, Fibα 580–600, Eno 5–21 (CEP-1), Fib α621–635, Fib α36–50, Fibβ 60–74, Ptm13, Ptm36, Pept-Z1, Pept-Z2, Pept-1, Pept-5, and Bla26. All 17 autoantibodies are described in more detail in ref. 18, except for Ptm13 and Ptm36, which are described in refs. 25 and 26 (details on the correlation structure are provided in Supplementary Table 1 and Supplementary Figure 1, available on the Arthritis & Rheumatology website at http://onlinelibrary.wiley.com/doi/10.1002/art.41381/abstract). The median number of ACPA subspecificities expressed was 6 (IQR 11). We noted borderline or statistically significant associations between the number of subspecificities expressed and the risk of each of the CV end points. The HRs for each CV end point per 1-unit increase in the number of specific ACPAs were as follows: for ACS, HR 1.02 (95% CI 0.99–1.05); for stroke, HR 1.04 (95% CI 1.01–1.07); for CV-related death, HR 1.04 (95% CI 1.00–1.08); and for MACE, HR 1.03 (95% CI 1.01–1.05) (Table 3).

Similar to the analyses of anti-CCP2 levels, we noted a trend toward increasing HRs for each CV end point with increasing autoantibody load (Table 3). The Spearman's rank correlation between anti-CCP2 level and number of ACPA subspecificities expressed was 0.89 ($P < 2.2 \times 10^{-16}$).

No single ACPA subspecificity was associated with all 4 CV outcomes (Table 4). In analyses of the 95% CIs of each HR, only anti–CEP-1 displayed a statistically significant association with the risk of ACS. With regard to risk of stroke, 10 subspecificities were significantly associated with this CV end point, with Fibβ 60–74 having the highest HR for association (HR 1.82, 95% CI 1.28–2.59). With regard to CV-related death and MACE, 5 autoantibodies and 9 autoantibodies, respectively, were significantly associated with the risk of these CV end points. After Bonferroni's correction for multiple hypothesis testing, all of these associations with individual subspecificities lost their statistical significance.

**RF isotypes.** For associations of RFs with CV end points, the pattern was markedly different across isotypes. For IgM-RF, risk associations with each of the CV outcomes were of a similar magnitude, but IgM-RF was significantly associated only with the risk of stroke (HR 1.42, 95% CI 1.01–2.01) and MACE (HR 1.40, 95% CI 1.11–1.76), but not with ACS (HR 1.40, 95% CI 1.00–1.96) or CV-related death (HR 1.47, 95% CI 0.94–2.27) (Table 4). IgA-RF was associated with an increased risk of CV-related death (HR 1.88, 95% CI 1.22–2.88) (Table 4). IgG-RF was not significantly associated with any of the CV outcomes (Table 4).

In the model with all 3 isotypes present, all of these associations with CV outcomes remained, but when anti-CCP2 status was included in the model, all associations (with the RF isotypes as well as with anti-CCP2) disappeared, except the association between IgA-RF and CV-related death (HR 1.92, 95% CI 1.04–3.56).

**Results of exploratory analysis.** All but 9 individuals had provided information about smoking habits at the time of RA diagnosis. When estimating the sex- and age-adjusted mortality rates for smokers and nonsmokers in the antibody-positive and antibody-negative groups, we observed that the high relative risks of CV-related death associated with IgA-RF and IgG-RF were attributable to the very low CV-related death rates observed in RF-negative never smokers (compared to the total incidence of CV-related deaths of 2.5 per 1,000 person-years) (Table 1). In the IgG-RF–negative patients who were never smokers, the rate of CV-related deaths was 0.40 (95% CI 0.08–1.17) per 1,000 person-years, and in IgA-RF–negative patients who were never smokers, it was 0.44 (95% CI 0.09–1.30) per 1,000 person-years (see Supplementary Table 3, available on the Arthritis & Rheumatology website at http://onlinelibrary.wiley.com/doi/10.1002/art.41381/abstract).

With regard to associations with the presence and/or titers of anti-CCP2, when analyses were adjusted for smoking status, the observed univariate associations with anti-CCP2 lost their statistical significance. In analyses stratified by both anti-CCP2 and smoking status, the only association remaining was between anti-CCP2 and risk of ACS (HR 1.51, 95% CI 1.01–2.27) among ever smokers.

With regard to associations with the RF isotypes, adjustment for smoking led to a decrease in the strength of all associations, but the association between IgA-RF and CV-related death (HR 1.61, 95% CI 1.05–2.48), and between IgM-RF and MACE (HR 1.28, 95% CI 1.01–1.62) remained significantly increased. In analyses stratified by smoking status, we observed associations between IgA-RF and IgG-RF and CV-related death and between IgG-RF and IgM-RF and MACE, among never smokers (see Supplementary Table 2, available on the Arthritis & Rheumatology website at http://onlinelibrary.wiley.com/doi/10.1002/art.41381/abstract). With regard to CV-related death among never smokers, these associations were notably elevated (for association with IgA-RF, HR 3.88, 95% CI 1.04–14.54; for association with IgG-RF, HR 6.29, 95% CI 1.63–24.25).

With regard to associations with most of the ACPA subspecificities, adjustment for smoking led to a decrease in the HRs. However, for the risk of CV-related death, the HRs for several autoantibodies were elevated. Nevertheless, similar to that in the main analysis, the statistical significance of these associations was lost following Bonferroni's correction.

In total, 1,762 individuals (63%) had information on smoking, income, and initial RA disease activity. Smoking was itself

**Table 3.** Association between presence and load of ACPA antibody subspecificities and incident ACS, stroke, CV-related death, and MACE among 2,814 Swedish patients followed up from RA diagnosis for incident CV events*

| | % of positive patients | ACS† | | Stroke‡ | | CV-related death | | MACE§ | |
|---|---|---|---|---|---|---|---|---|---|
| | | HR (95% CI) | *P* | HR (95% CI) | *P* | HR (95% CI) | *P* | HR (95% CI) | *P* |
| Any ACPA subspecificities | 6 (11)¶ | 1.02 (0.99–1.05) | 0.11 | 1.04 (1.01–1.07) | 0.007 | 1.04 (1.00–1.08) | 0.058 | 1.03 (1.01–1.05) | 0.0026 |
| ACPA subspecificity load | | | | | | | | | |
| Low (1–4) | 24 | 1.38 (0.82–2.31) | 0.22 | 1.62 (0.93–2.83) | 0.088 | 1.01 (0.51–1.99) | 0.97 | 1.32 (0.92–1.88) | 0.13 |
| Medium (5–10) | 25 | 1.51 (0.91–2.52) | 0.11 | 1.95 (1.14–3.34) | 0.015 | 1.42 (0.75–2.69) | 0.28 | 1.54 (1.09–2.18) | 0.014 |
| High (11–17) | 31 | 1.60 (0.99–2.57) | 0.053 | 2.14 (1.28–3.57) | 0.0035 | 1.59 (0.89–2.85) | 0.12 | 1.67 (1.21–2.31) | 0.002 |
| *P* for trend | – | – | 0.057 | – | 0.0032 | – | 0.066 | – | 0.0014 |

* Values for the association between the presence and load of anti–citrullinated protein antibody (ACPA) subspecificities and incident acute coronary syndrome (ACS), stroke, cardiovascular (CV)–related death, and major adverse CV events (MACE) are shown as the hazard ratio (HR) with 95% confidence interval (95% CI).
† Adjusted for age, sex, age$^2$, and calendar period of rheumatoid arthritis (RA) diagnosis and stroke event before the diagnosis.
‡ Adjusted for age, sex, age$^2$, and calendar period of RA diagnosis and ACS event before the diagnosis.
§ Adjusted for age, sex, age$^2$, and calendar period of RA diagnosis.
¶ Values are the median number (interquartile range) of ACPA subspecificities expressed.

**Table 4.** Association between specific ACPA subspecificities and RF isotypes and incident ACS, stroke, CV-related death, and MACE among 2,814 Swedish patients followed up from RA diagnosis for incident CV events*

| | % of positive patients | ACS† | | Stroke‡ | | CV-related death | | MACE§ | |
|---|---|---|---|---|---|---|---|---|---|
| | | HR (95% CI) | *P* | HR (95% CI) | *P* | HR (95% CI) | *P* | HR (95% CI) | *P* |
| ACPA subspecificity | | | | | | | | | |
| Fil 307–324 (CCP-1) | 43 | 1.33 (0.96–1.85) | 0.082 | 1.42 (1.02–1.98) | 0.037 | 1.14 (0.75–1.74) | 0.54 | 1.33 (1.06–1.66) | 0.012 |
| Vim 60–75 | 46 | 1.10 (0.80–1.53) | 0.56 | 1.51 (1.08–2.10) | 0.015 | 1.37 (0.90–2.09) | 0.14 | 1.28 (1.02–1.59) | 0.033 |
| Vim 2–17 | 33 | 1.22 (0.87–1.71) | 0.24 | 1.11 (0.78–1.57) | 0.57 | 1.28 (0.82–1.98) | 0.27 | 1.18 (0.94–1.49) | 0.16 |
| Fibβ 36–52 | 47 | 1.37 (0.99–1.90) | 0.056 | 1.36 (0.98–1.90) | 0.068 | 1.30 (0.85–1.99) | 0.22 | 1.34 (1.07–1.68) | 0.0098 |
| Fibα 563–583 | 41 | 1.08 (0.78–1.51) | 0.63 | 1.45 (1.04–2.03) | 0.028 | 1.30 (0.85–1.99) | 0.23 | 1.23 (0.98–1.54) | 0.068 |
| Fibα 580–600 | 25 | 1.32 (0.93–1.87) | 0.12 | 1.33 (0.93–1.91) | 0.12 | 1.31 (0.83–2.07) | 0.25 | 1.37 (1.08–1.74) | 0.011 |
| Eno 5–21 (CEP-1) | 47 | 1.41 (1.02–1.96) | 0.037 | 1.40 (1.00–1.95) | 0.048 | 1.12 (0.74–1.72) | 0.59 | 1.32 (1.06–1.65) | 0.015 |
| Fibα 621–635 | 35 | 1.29 (0.93–1.80) | 0.13 | 1.48 (1.06–2.07) | 0.021 | 1.11 (0.72–1.73) | 0.63 | 1.23 (0.98–1.55) | 0.071 |
| Fibα 36–50 | 17 | 1.15 (0.77–1.73) | 0.49 | 1.63 (1.11–2.39) | 0.012 | 1.09 (0.63–1.87) | 0.77 | 1.41 (1.08–1.84) | 0.012 |
| Fibβ 60–74 | 58 | 1.18 (0.85–1.64) | 0.32 | 1.82 (1.28–2.59) | 0.00096 | 1.66 (1.07–2.58) | 0.025 | 1.44 (1.14–1.81) | 0.0019 |
| Ptm13 | 11 | 1.46 (0.95–2.25) | 0.086 | 0.82 (0.47–1.42) | 0.47 | 1.05 (0.55–1.97) | 0.89 | 1.17 (0.85–1.62) | 0.34 |
| Ptm36 | 28 | 1.14 (0.80–1.63) | 0.46 | 1.16 (0.81–1.66) | 0.41 | 1.28 (0.81–2.01) | 0.28 | 1.16 (0.91–1.47) | 0.24 |
| Pept-Z1 | 53 | 1.14 (0.82–1.57) | 0.45 | 1.36 (0.97–1.90) | 0.073 | 1.69 (1.10–2.61) | 0.017 | 1.25 (1.00–1.56) | 0.051 |
| Pept-Z2 | 41 | 1.20 (0.86–1.66) | 0.28 | 1.36 (0.97–1.89) | 0.072 | 1.61 (1.06–2.46) | 0.026 | 1.25 (1.00–1.56) | 0.053 |
| Pept-1 | 34 | 1.02 (0.72–1.43) | 0.92 | 1.44 (1.02–2.02) | 0.036 | 1.58 (1.03–2.43) | 0.036 | 1.22 (0.97–1.54) | 0.09 |
| Pept-5 | 54 | 1.23 (0.88–1.70) | 0.22 | 1.58 (1.12–2.23) | 0.0096 | 1.55 (1.00–2.39) | 0.05 | 1.36 (1.08–1.71) | 0.0078 |
| Bla-26 | 32 | 1.22 (0.87–1.71) | 0.25 | 1.46 (1.04–2.05) | 0.031 | 1.55 (1.01–2.38) | 0.047 | 1.34 (1.07–1.69) | 0.012 |
| RF isotype | | | | | | | | | |
| IgA-RF | 42 | 1.13 (0.81–1.56) | 0.48 | 1.10 (0.79–1.53) | 0.59 | 1.88 (1.22–2.88) | 0.0038 | 1.17 (0.94–1.47) | 0.16 |
| IgG-RF | 32 | 1.17 (0.84–1.64) | 0.36 | 1.23 (0.87–1.73) | 0.25 | 1.40 (0.91–2.16) | 0.13 | 1.15 (0.91–1.46) | 0.23 |
| IgM-RF | 57 | 1.40 (1.00–1.96) | 0.052 | 1.42 (1.01–2.01) | 0.045 | 1.47 (0.94–2.27) | 0.088 | 1.40 (1.11–1.76) | 0.0045 |

* Values for the association between specific anti–citrullinated protein antibody (ACPA) subspecificities and rheumatoid factor (RF) isotypes and incident acute coronary syndrome (ACS), stroke, cardiovascular (CV)–related death, and major adverse CV events (MACE) are shown as the hazard ratio (HR) with 95% confidence interval (95% CI).
† Adjusted for age, sex, age$^2$, and calendar period of rheumatoid arthritis (RA) diagnosis and stroke event before the diagnosis.
‡ Adjusted for age, sex, age$^2$, and calendar period of RA diagnosis and ACS event before the diagnosis.
§ Adjusted for age, sex, age$^2$, and calendar period of RA diagnosis.

associated with all CV-related outcomes except ACS. Income level, but not the initial DAS28 value, was associated with all outcomes except stroke. In this patient subset of 1,762 patients, the observed overall associations with anti-CCP2 positivity, the trends toward association with anti-CCP2 levels, the ACPA autoantibody load, and the trends toward associations with ACPA autoantibody load all remained notable or were accentuated in comparison to the associations observed in analyses of the entire cohort of 2,814 patients. Adjustment of these analyses for smoking status, income level, and DAS28 scores only slightly shifted the HRs, although the statistical significance of some of the trends was lost in this subset of patients comprising 63% of the original population. With regard to IgA-RF, the HR for CV-related death was 2.65 (95% CI 1.51–4.63), and this association remained significant when smoking status, income level, and DAS28 scores were included in the model (HR 2.35, 95% CI 1.34–4.14) and also when the model was adjusted for anti-CCP2 status (HR 2.87, 95% CI 1.19–6.93). Results of all of these analyses are shown in Supplementary Tables 4, 5, 6, and 7, available on the Arthritis & Rheumatology website at http://onlinelibrary.wiley.com/doi/10.1002/art.41381/abstract.

When the outcome was changed to all-cause mortality, we noted strong associations with anti-CCP2 positivity, anti-CCP2 level, autoantibody load, most of the ACPA subspecificities, and each RF isotype (see Supplementary Table 8, available on the Arthritis & Rheumatology website at http://onlinelibrary.wiley.com/doi/10.1002/art.41381/abstract).

## DISCUSSION

We investigated the association between ACPA positivity and levels, ACPA subspecificities, RF isotypes, and incident CV events in a large and prospectively monitored inception cohort of patients with RA. We made the following important observations: 1) ACPA (anti-CCP2) positivity in RA is a marker for several CV end points. 2) These associations trended toward an association with higher anti-CCP2 levels. 3) A trend of association of the CV end points with increasing number of ACPA subspecificities expressed was also evident. 4) Whereas many individual ACPAs were associated with the CV end points, there was no clear pattern of particularly strong associations with any particular ACPA, at least not consistently so within specific groups of ACPAs targeting citrullinated epitopes in individual proteins across CV end points. 5) Presence of RF was associated with CV event risk, but the associations differed across the RF isotypes, with IgM-RF being associated with stroke and MACE, but IgA-RF being linked to CV-related mortality. 6) Finally, we noted that for most of the autoantibodies, the associations decreased or disappeared when the analyses were adjusted for smoking status, initial RA disease activity, and socioeconomic status, whereas with regard to IgA-RF, the association with CV-related death remained.

With regard to ACPA positivity per se, our results corroborate but also extend those of previous studies on RA serologic markers and RA outcomes. For instance, Ajeganova et al compared the association between the presence of anti-CCP2 or RF and mortality across 3 RA cohorts, and found associations with CV-related mortality with an effect size similar to ours (27). In our present study, we expanded these findings by also assessing the levels of anti-CCP2, as well as 17 different ACPA subspecificities and IgA- and IgG-RF. We further demonstrated that the associations of these autoantibodies applied across several CV clinical fatal or nonfatal phenotypes, rather than being merely markers of increased overall mortality (which is also a recognized role of these autoantibodies, as demonstrated by our exploratory analyses).

In contrast to previous studies, we further investigated different subspecificities of ACPAs. The mechanistic and pathologic roles of the different subspecificities are unclear, and our epidemiologic investigation was not designed to address such questions. Interestingly, a previous report on citrullinated antibodies and associations with atherosclerotic plaques in early RA (14) found the presence of both citrullinated vimentin and citrullinated fibrinogen in the plaques, as well as an association between the presence of anticitrullinated vimentin and fibrinogen antibodies and increased plaque burden. We aimed to elucidate whether any particular subspecificity carried a greater risk, indicating that it might be more causally involved, but such attempts are difficult to design and interpret, and we fully acknowledge the complexity of the data, with the intricate structure of co-reactivity and potential cross-reactivity between the different ACPA subspecificities. Although we did find that many subspecificities were associated with different types of CV end points, subspecificities targeting a specific protein were never associated with the same outcome. What seemed more important, and remained consistent in the exploratory analysis, was the number of subspecificities expressed, which is also consistent with the observation of a strong correlation between anti-CCP2 level and number of subspecificities expressed.

With regard to RF, several reports have described an association with overall mortality (28–30), overall CV mortality (27,30), and with certain CV phenotypes (31) in RA, which has also been the case in the general (non-RA) population (15,16). Our results corroborate (with respect to RF overall) and extend (with respect to isotype-specific results) these findings. It should be noted, though, that the previously reported associations between RF and CV disease in the general population are difficult to interpret, as the prevalence of RF in both of the existing studies were higher than expected (15,16). This might be attributable to the only existing instruction denoting a cutoff level for RA-associated autoantibodies, the open-ended cutoff of >95% specificity for RF described in the ACR 1987 classification criteria for RA (19). For ACPAs, including anti-CCP2, no parallel directions exist, and cutoff levels in fact vary more than 500% between different commercial tests (see Table 2 in ref. 32). For that reason, we carefully

adjusted all autoantibody cutoff levels to the 98th percentile, which is close to the 98.4% specificity for anti-CCP2 in the EIRA study. Our study thus further extends the results by demonstrating how these associations vary across RF isotypes, where IgM-RF was associated with several CV disease types, but IgA-RF was more distinctly associated with CV-related mortality. One explanation could be that an association between IgA-RF and extraarticular manifestations was previously observed in patients with RA (33), whose mortality rate was increased and in whom CV events were the main cause of death (34).

Our study has some limitations. Because of missing data, the subset of patients studied, in which adjustments could be made for smoking status and other risk factors, was smaller than the original study population (but displayed very similar associations). We further had limited means to adjust for certain CV risk factors such as lipid profile, family history of CV disease, physical activity, and certain comorbid conditions, such as hypothyroidism, which, by themselves, may or may not affect CV risk. We also did not have data to address the impact of the disease course on the outcomes. Instead, we designed our study to investigate the association between serologic status at baseline and CV outcomes.

Strengths of our study include the identification of, and prospective, complete, and independent follow-up of a large cohort of patients newly diagnosed as having RA (the largest studied so far among studies of fine specificities and CV outcomes in patients with RA) who were treated in rheumatology clinics according to standard treatment guidelines. Our CV end points were all based on validated and robust definitions. Moreover, we were able to centrally analyze the serum samples obtained at baseline, thereby minimizing interassay/interreader variability, selection bias, or reversed causality. In addition, we could assess the potential impact of certain additional CV risk factors, although the size of the study and the time period covered prevented accurate identification of additional risk factors, such as diabetes, across the entire RA cohort. Because of the robustness of the data, we also believe our findings to be generalizable to other countries.

From a clinical perspective, our data imply that patients with seronegative RA are at a lower risk of CV events, while patients with high anti-CCP2 levels or presence of IgA- or IgG-RF at diagnosis are at higher risk and might benefit from closer monitoring from a cardio-preventative perspective. In this regard, specific ACPA subspecificities offer no further predictive capacity.

In conclusion, patients with RA who are seropositive for ACPAs and RF carry a risk of several types of CV end points, including CV-related and overall mortality, which is independent of a history of ever smoking. These associations do not seem to be driven by any specific (pattern of) ACPAs, suggesting that for CV comorbidities in RA, autoantibody load may be more important than individual ACPAs. For RF, the risks of CV events seem to vary across RF isotypes.

## AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Westerlind had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Acquisition of data.** Rönnelid, Hansson, Alfredsson, Mathsson-Alm, Serre, Cornillet, Holmdahl, Jakobsson, Skriner, Klareskog, Saevarsdottir, Askling.
**Analysis and interpretation of data.** Westerlind, Rönnelid, Hansson, Alfredsson, Mathsson-Alm, Serre, Cornillet, Holmdahl, Jakobsson, Skriner, Klareskog, Saevarsdottir, Askling.

## ADDITIONAL DISCLOSURES

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