

Autoantibodies and the Risk of Cardiovascular Events

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ABSTRACT. *Objective.* Inflammation and autoimmunity are associated with increased cardiovascular (CV) risk in patients with rheumatoid arthritis. This association may also be present in those without rheumatic diseases. Our purpose was to determine whether rheumatoid factor (RF), antinuclear antibody (ANA), and cyclic citrullinated peptide antibody (CCP) positivity are associated with increased risk of CV events and overall mortality in those with and without rheumatic diseases.

Methods. We performed a population-based cohort study of all subjects who had a RF and/or ANA test performed between January 1, 1990, and January 1, 2000, and/or CCP test performed between September 1, 2003, and January 1, 2005, with followup until April 1, 2007. Outcomes were ascertained using diagnostic indices from complete medical records, including CV events [myocardial infarction (MI), heart failure (HF), and peripheral vascular disease (PVD)] and mortality. Cox models were used to analyze the data.

Results. There were 6783 subjects with RF, 7852 with ANA, and 299 with CCP testing. Of these, 10.4%, 23.9%, and 14.7% were positive for RF, ANA, and CCP, respectively. Adjusting for age, sex, calendar year, comorbidity, and rheumatic disease, RF and ANA positivity were significant predictors of CV events [hazard ratio (HR) 1.24 and 1.26] and death (HR 1.43 and 1.18). Adjusting for age, CCP positivity was associated with CV events, but this association was not statistically significant (HR 3.1; 95% CI 0.8, 12.3).

Conclusion. RF and ANA positivity are significant predictors of CV events and mortality in both those with and those without rheumatic diseases. These results support the role of immune dysregulation in the etiology of CV disease. (J Rheumatol First Release Oct 15 2009; doi:10.3899/jrheum.090188)

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Understanding of the pathogenesis of cardiovascular (CV) disease entered a new era since the recognition of the role of inflammation in atherothrombosis^{1,2}. The bulk of the evidence to date suggests that atherosclerosis is in large part a

chronic inflammatory disease that can manifest with an acute clinical event by plaque rupture and thrombosis.

It has long been recognized that patients with systemic inflammatory autoimmune diseases are at increased risk of CV disease^{3,4}. Moreover, disease-related factors such as markers of systemic inflammation are associated with CV events in such patients^{5,6}. Another disease-related factor that may mediate atherosclerosis is the presence of autoimmunity or immune dysregulation. Indeed, patients with rheumatoid arthritis (RA) who are seropositive for rheumatoid factor (RF) have a higher risk of CV events and mortality than seronegative patients with RA⁷. An intriguing question is whether autoimmunity, as evidenced by presence of autoantibodies, may be associated with increased CV risk not only in those with rheumatic diseases, but also in patients with seropositivity but without clinical rheumatic disease activity. This is especially important because a better understanding of the role of autoimmunity in CV disease may have prognostic implications, and may clinically affect CV disease prediction and prevention strategies for both patients with and those without rheumatic diseases.

The purpose of our study was to determine the relationship between the commonly tested autoantibodies RF and antinuclear antibody (ANA) and the risk of CV events in individuals with and without rheumatic diseases. We also

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explored the association between cyclic citrullinated peptide (CCP) antibody and the risk of CV events in those with and without rheumatic diseases. A secondary objective was to examine the possible effect of confounding by indication for autoantibody testing on these results.

MATERIALS AND METHODS

Data collection. Using the resources of the Rochester Epidemiology Project⁸, we identified all Olmsted County residents who had RF and/or ANA testing between January 1, 1990, and January 1, 2000, and/or CCP testing between September 1, 2003, and January 1, 2005 (due to availability of CCP testing at our institution). All subjects were followed up until April 1, 2007. The institutional review boards of the Mayo Foundation and Olmsted Medical Center approved this study.

Data were transcribed from the medical record or electronically retrieved, including the RF, CCP, and ANA test results; dates of first tests; and dates of first positive tests within the respective timeframes for all subjects. If a patient had multiple testing within the respective timeframes, only the date and result of the first positive test in the timeframe were analyzed.

The test results were separated into positive or negative to account for changes in reference ranges and testing methods over time. RF testing was performed by nephelometry (Beckman Auto ICS system, Beckman Coulter, Fullerton, CA, USA) for mostly IgM RF, or latex agglutination assay (Dade RapiTex kit) for IgG RF. ANA testing was performed by immunofluorescence using HEp-2 cells (Kallestad HEp-2 Kit, Bio-Rad Laboratories, Hercules, CA, USA) or ELISA using a HEp-2 cell lysate as the antigen source (ANA Screening Test, Bio-Rad Laboratories). Anti-CCP testing was performed by enzyme immunoassay (Diastat Anti-CCP EIA, Axis-Shield, Dundee, UK). Positive results were defined by the reported clinical laboratory standards, as follows: RF positivity was ≥ 40 IU/ml or a semiquantitative titer of 1:80 or greater, ANA positivity was ≥ 1.0 U or a titer of 1:40 or greater, and CCP positivity was > 5.0 U/ml. In addition, for RF, “weak positives” were defined as 40–79 IU/ml or a semiquantitative titer of 1:80, and “strong positives” were defined as ≥ 80 IU/ml or a titer of 1:160 or greater. For ANA, weak positives were defined as 1.0–3.0 U or a titer between 1:40 and 1:160, and strong positives were defined as ≥ 3.0 U or a titer of 1:320 or greater.

CV outcomes and comorbid conditions were ascertained using the electronic indices of diagnoses [*International Classification of Diseases*, 9th ed (ICD-9) codes] recorded from the complete (inpatient and outpatient) community medical records (see Appendix for ICD-9 code list). Cardiovascular outcomes included myocardial infarction (MI), heart failure (HF), peripheral vascular disease (PVD), and overall mortality. Comorbid conditions included rheumatic diseases, infections (including those that may produce false-positive RF tests), nonrheumatic autoimmune diseases (including autoimmune thyroid, liver, and pulmonary disease; see Appendix), diabetes mellitus, cerebrovascular disease, dementia, chronic pulmonary disease, peptic ulcer disease, liver disease, renal disease, and malignancy^{9,10}. Rheumatic disease diagnoses included rheumatoid arthritis (RA), polymyalgia rheumatica (PMR), systemic lupus erythematosus (SLE), and other connective tissue diseases (CTD). Vital status was ascertained by utilizing death certificates and the medical records, as described⁶.

The presence of comorbid conditions and CV outcomes was recorded as present at baseline (i.e., at time of testing) or date of first (incident) diagnosis during followup until April 1, 2007. In addition to diabetes mellitus, information on additional CV risk factors including presence of high blood pressure, high cholesterol, smoking (ever vs never), and alcohol use (ever vs never) was also ascertained electronically from the medical record.

To address the secondary aim of examining potential confounding by indication for autoantibody testing, 3 random samples of subjects who did not receive a RF, CCP, or ANA test were selected from among those who received medical care in Olmsted County, matched electronically on age,

sex, and length of medical record to respective sample groups of subjects tested for the respective autoantibodies. Presence of comorbid conditions and rheumatic diseases was ascertained electronically in both the groups of subjects who did and the control groups who did not receive autoantibody testing.

Statistical analysis. We used Cox proportional hazards models to test the hypotheses that RF, ANA, and CCP positivity are associated with an increased risk of CV events and mortality. Patients with CV events prior to autoantibody testing were excluded from these analyses. The combined event “MI, HF, or PVD” was defined as the first occurrence of any of the 3 events. All models were started 6 months after the first autoantibody test within the timeframe. Multivariable Cox models were used to examine the effect of RF, ANA, and CCP positivity after adjusting for age, sex, calendar year, chronic disease comorbidities, and the presence of rheumatic disease. The development of rheumatic diseases was tracked through each subject’s total followup as a time-dependent covariate, taking advantage of our extensive surveillance of the population.

Additionally, to examine the robustness of our findings, we performed the above analyses for “weak” and “strong” RF and ANA positivity, as well as in the absence of rheumatic disease, i.e., with subjects censored at diagnosis of rheumatic disease. In additional analyses we also adjusted for other CV risk factors including high blood pressure, high cholesterol, smoking, and alcohol use, in the subset of patients for whom these data were available electronically.

In supplementary analyses to address the potential bias of confounding by indication, we used Cox proportional hazards models to examine the potential effect of being tested for RF or ANA by comparing the development of CV outcomes in a 10% random sample of individuals tested for RF or ANA compared to individuals matched by age, sex, and length of medical record who were not tested for RF or ANA, respectively. Two controls who were not tested were matched to each of the tested patients. Distributions of demographics and characteristics of those tested and not tested were compared using chi-squared tests. Multivariable models were again adjusted for age, sex, calendar year, chronic disease comorbidities, and presence of rheumatic disease.

RESULTS

There were 6783 subjects who underwent RF testing, 7852 with ANA testing and 299 with CCP testing. Table 1 shows the demographics, followup, and percentages of subjects tested positive for autoantibodies and those who had or who developed RA, SLE, PMR, and other CTD, at time of testing or over time, as well as the percentages of patients with positive tests for autoantibodies who developed rheumatic diseases. The mean length of followup was 9.4 years for RF, 9.2 years for ANA, and 2.5 years for CCP-tested individuals.

Table 2 shows the effect of positivity for RF or ANA on the risk of CV outcomes and death, in multivariable models both before and after adjustment for the presence of rheumatic diseases. After adjusting for age, sex, calendar year, and comorbidities, a positive RF test was a significant predictor of MI, HF, or PVD [hazard ratio (HR) 1.32, 95% confidence interval (CI) 1.10, 1.59] and death (HR 1.55, 95% CI 1.33, 1.80). After further adjustment for the presence of rheumatic diseases, RF positivity remained a significant predictor of MI, HF, or PVD (HR 1.24, 95% CI 1.01, 1.51) and a strong predictor of death (HR 1.43, 95% CI 1.21, 1.68). Most of the increased risk of CV outcomes appeared to result from an increased risk of MI and HF (Table 2).

We also analyzed the risk of CV outcomes associated

Table 1. Characteristics of subjects who received autoantibody testing.

Characteristic	RF	Autoantibody ANA	CCP
No. subjects tested	6783	7852	299
No. positive (%)	703 (10.4)	1877 (23.9)	44 (14.7)
Female, n (%)	4706 (69.4)	5408 (68.8)	212 (70.9)
Age at first test (mean ± SD), yrs	49.7 ± 17.0	47.5 ± 17.0	54.5 ± 15.8
Length of followup (mean ± SD), yrs	9.4 ± 4.9	9.2 ± 5.0	2.5 ± 0.8
Patients who had rheumatic diseases (at time of testing or over time)			
Rheumatoid arthritis, n (%)	831 (12.2)	591 (7.5)	158 (52.8)
Systemic lupus erythematosus, n (%)	114 (1.7)	159 (2.0)	15 (5.0)
Polymyalgia rheumatica, n (%)	246 (3.6)	190 (2.4)	28 (9.4)
Other connective tissue disease, n (%)	114 (1.7)	130 (1.7)	18 (6.0)
Any of the above rheumatic diseases, n (%)	1147 (16.9)	943 (12.0)	182 (60.9)
Patients who had rheumatic diseases (among those with positive tests)			
Any of the above rheumatic diseases, n (%)	456 (64.9)	431 (23.0)	42 (95.4)

RF: rheumatoid factor; ANA: antinuclear antibody; CCP: cyclic citrullinated peptide antibody.

Table 2. Risk of autoantibody positivity on cardiovascular outcomes and death.

Autoantibody	Outcome	Events	Hazard Ratio (95% CI)	
			Unadjusted for Presence of Rheumatic Disease*	Adjusted for Presence of Rheumatic Disease*
RF	MI	365	1.36 (1.03, 1.79)	1.22 (0.91, 1.64)
	HF	499	1.47 (1.17, 1.84)	1.36 (1.07, 1.73)
	PVD	507	1.13 (0.88, 1.46)	1.08 (0.82, 1.43)
	MI, HF, or PVD**	871	1.32 (1.10, 1.59)	1.24 (1.01, 1.51)
	Death	998	1.55 (1.33, 1.80)	1.43 (1.21, 1.68)
ANA	MI	371	1.32 (1.06, 1.65)	1.29 (1.03, 1.61)
	HF	502	1.13 (0.93, 1.38)	1.11 (0.92, 1.35)
	PVD	522	1.37 (1.13, 1.66)	1.35 (1.12, 1.64)
	MI, HF, or PVD**	890	1.28 (1.10, 1.48)	1.26 (1.09, 1.46)
	Death	1142	1.19 (1.05, 1.35)	1.18 (1.04, 1.34)

* Adjusted for age, sex, calendar year, comorbidity. ** Defined as first occurrence of any of the 3 events. RF: rheumatoid factor; ANA: antinuclear antibody; MI: myocardial infarction; HF: heart failure; PVD: peripheral vascular disease; comorbidity: Charlson⁹ category comorbidities.

with weakly positive and strongly positive RF. Only strongly positive RF conferred an excess risk of MI, HF, or PVD (HR 1.42, 95% CI 1.13, 1.79) and death (HR 1.62, 95% CI 1.35, 1.93), which was significant even after adjusting for the presence of rheumatic diseases (Table 3). There was no increased CV risk associated with weakly positive RF, either before or after adjustment for rheumatic disease (Table 3).

ANA positivity was also a significant predictor of MI, HF, or PVD (HR 1.28, 95% CI 1.10, 1.48) and death (HR 1.19, 95% CI 1.05, 1.35) (Table 2). After further adjustment for the presence of rheumatic diseases, ANA positivity still remained a significant predictor of CV events and death, but most of the increased risk of CV outcomes appeared to result from an increased risk of MI and PVD (Table 2).

In contrast to the analyses of weak and strong positive RF, we found an increased risk of CV outcomes from both weak and strong ANA positivity (Table 3). There was a significantly increased risk of MI, HF, or PVD (HR 1.23, 95%

CI 1.06, 1.43) and death (HR 1.17, 95% CI 1.03, 1.33) from weakly positive ANA, which remained significant after adjustment for the presence of rheumatic diseases. After adjustment for rheumatic diseases, there was an almost 2-fold increased risk of MI, HF, or PVD associated with strongly positive ANA. There was also a trend towards a higher risk of death (HR 1.31, 95% CI 0.82, 2.07), although the small numbers of patients with strongly positive ANA led to wider CI for this outcome.

Of note, for both ANA and RF, when adjustment for comorbidities was expanded to include infections and non-rheumatic autoimmune disorders, the results remained essentially unchanged (data not shown). In addition, after further adjustment for other CV risk factors, the results again remained essentially unchanged (risk of MI, HF, or PVD associated with RF, HR 1.26, 95% CI 0.96, 1.65; and associated with ANA, HR 1.25, 95% CI 1.02, 1.52), but the CI were slightly wider, as expected following these additional adjustments.

Table 3. Risk of “weak” versus “strong” autoantibody positivity on cardiovascular outcomes and death.

Autoantibody	Outcome	Hazard Ratio (95% CI)	
		Unadjusted for Presence of Rheumatic Disease*	Adjusted for Presence of Rheumatic Disease*
Weak [†] RF	MI, HF, or PVD**	0.93 (0.64, 1.36)	0.90 (0.61, 1.31)
	Death	1.04 (0.76, 1.43)	0.98 (0.71, 1.34)
Strong [†] RF	MI, HF, or PVD**	1.53 (1.24, 1.89)	1.42 (1.13, 1.79)
	Death	1.75 (1.48, 2.07)	1.62 (1.35, 1.93)
Weak ^{††} ANA	MI, HF, or PVD**	1.24 (1.07, 1.44)	1.23 (1.06, 1.43)
	death	1.19 (1.04, 1.35)	1.17 (1.03, 1.33)
Strong ^{††} ANA	MI, HF, or PVD**	1.90 (1.21, 2.98)	1.87 (1.19, 2.94)
	Death	1.34 (0.85, 2.13)	1.31 (0.82, 2.07)

* Adjusted for age, sex, calendar year, comorbidity. ** Defined as first occurrence of any of the 3 events. † Weak positive RF = 1:80 or 40–79 IU/ml; strong positive RF = 1:160 or ≥ 80 IU/ml; HR for weak and strong positive compared to normal RF. †† Weak positive ANA = 1:40 to 1:160 or 1–3 U; strong positive ANA = 1:320 or ≥ 3 U; HR for weak and strong positive compared to normal ANA. . RF: rheumatoid factor; ANA: antinuclear antibody; MI: myocardial infarction; HF: heart failure; PVD: peripheral vascular disease; comorbidity: chronic disease comorbidities⁹.

We performed additional analyses on the risk of CV outcomes associated with positivity for RF and ANA in the absence of rheumatic disease, i.e., with subjects censored at diagnosis of rheumatic disease. These results were essentially unchanged from the data presented in Table 2 (data not shown).

Due to the short followup time and small numbers of events among those tested for CCP, analyses regarding risk of CV outcomes in CCP-positive individuals were limited. After adjustment for age alone, CCP positivity was associated with MI, HF, or PVD but was not statistically significant (HR 3.11, 95% CI 0.8, 12.3), and it appeared to be a predictor of death (HR 7.89, 95% CI 1.8, 34.5). However, there were too few events and too short followup time to analyze the risk of CCP positivity on CV outcomes after adjustment for rheumatic diseases.

Subgroup analyses of the risk of CV events and death associated with RF and ANA positivity in men compared to women is shown in Table 4. After adjustment for age, calendar year, comorbidity, and presence of rheumatic diseases, the risk of MI, HF, or PVD was significantly increased in RF-seropositive men but not in women. In addition, we observed a significantly increased risk of death in both men and women associated with RF positivity. For

ANA positivity, there was a significantly increased risk of MI, HF, or PVD in both men and women, as well as death in women. Although not statistically significant, there was also a trend for increased risk of death in men with ANA positivity.

Finally, we examined the potential for confounding by indication for autoantibody testing. Table 5 shows the characteristics and chronic disease comorbidities in subjects tested for RF or ANA versus subjects who were not tested for RF or ANA, respectively. As expected, there was a higher proportion of rheumatic diseases in those tested for autoantibodies versus those not tested. However, the proportions of chronic disease comorbidities were similar between both groups. Table 6 shows the results of Cox proportional hazards models comparing the risk of CV events in individuals tested for RF or ANA versus control groups matched on age, sex, and length of medical record who were not tested for RF or ANA. There was no evidence for an increased risk of either CV events or death among those tested for RF or ANA compared to those not tested, after adjustment for age, sex, calendar year, comorbidity, and presence of rheumatic disease ($p > 0.16$ for all comparisons). These findings argue against the existence of significant confounding by indication.

Table 4. Risk of cardiovascular outcomes associated with autoantibodies in men versus women.

Autoantibody	Outcome	Events		Hazard Ratio (95% CI)*	
		Men	Women	Men	Women
RF	MI, HF, or PVD**	313	558	1.46 (1.04, 2.04)	1.14 (0.88, 1.46)
	Death	382	616	1.31 (1.00, 1.71)	1.56 (1.27, 1.92)
ANA	MI, HF, or PVD**	310	580	1.40 (1.07, 1.82)	1.22 (1.02, 1.45)
	Death	475	667	1.17 (0.95, 1.44)	1.20 (1.02, 1.41)

* Adjusted for age, calendar year, comorbidity, and presence of rheumatic disease. ** Defined as first occurrence of any of the 3 events. RF: rheumatoid factor; ANA: antinuclear antibody; MI: myocardial infarction; HF: heart failure; PVD: peripheral vascular disease; comorbidity: chronic disease comorbidities⁹.

Table 5. Characteristics of subjects with autoantibody testing vs control subjects without autoantibody testing.

Characteristic	RF		ANA	
	Subjects with AutoAb Testing	Subjects Without AutoAb Testing	Subjects with AutoAb Testing	Subjects Without AutoAb Testing
No.	664	1259	772	1470
Female, n (%)	457 (68.8)	868 (68.9)	539 (69.8)	1032 (70.2)
Age at first test/index (mean ± SD), yrs	50.0 ± 16.6	49.0 ± 16.5	47.8 ± 16.6	47.1 ± 16.6
Length of followup (mean ± SD), yrs	9.7 ± 4.7	9.6 ± 4.7	9.5 ± 4.7	9.5 ± 4.8
Chronic disease comorbidities at time of testing/index, n (%)				
Diabetes mellitus	46 (6.9)	70 (5.6)	45 (5.8)	70 (4.8)
Liver disease	11 (1.7)	20 (1.6)	21 (2.7)	24 (1.6)
Cerebrovascular disease	45 (6.8)	75 (6.0)	52 (6.7)	68 (4.6)*
Chronic pulmonary disease	97 (14.6)	182 (14.5)	115 (14.9)	188 (12.8)
Peptic ulcer	51 (7.7)	69 (5.5)	53 (6.9)	84 (5.7)
Renal disease	17 (2.6)	35 (2.8)	22 (2.8)	31 (2.1)
Malignancy	52 (7.8)	107 (8.5)	64 (8.3)	131 (8.9)
Patients who had rheumatic diseases (at time of testing/index or over time)				
Rheumatoid arthritis, n (%)	80 (12.0)	40 (3.2)**	53 (6.9)	47 (3.2)**
Systemic lupus erythematosus, n (%)	8 (1.2)	6 (0.5)	16 (2.1)	9 (0.6)**
Polymyalgia rheumatica, n (%)	21 (3.2)	17 (1.4)**	20 (2.6)	24 (1.6)
Other connective tissue disease, n (%)	12 (1.8)	3 (0.2)**	11 (1.4)	4 (0.3)**

* Those with testing compared to those without testing, $p < 0.01$, ** $p < 0.05$. RF: rheumatoid factor; ANA: antinuclear antibody.

Table 6. Risk of cardiovascular outcomes in subjects with autoantibody testing vs matched control subjects without autoantibody testing.

Autoantibody	Outcome	Events	Hazard Ratio (95% CI)*
RF	MI, HF, or PVD**	230	1.04 (0.78, 1.37)
	Death	264	0.95 (0.73, 1.24)
ANA	MI, HF, or PVD**	215	1.20 (0.93, 1.56)
	Death	218	0.93 (0.73, 1.19)

* Adjusted for age, sex, calendar year, comorbidity, and presence of rheumatic disease. ** Defined as first occurrence of any of the 3 events. RF: rheumatoid factor; ANA: antinuclear antibody; MI: myocardial infarction; HF: heart failure; PVD: peripheral vascular disease; HR: hazard ratio; comorbidity: chronic disease comorbidities⁹.

DISCUSSION

It has long been recognized that autoimmune diseases such as RA and SLE are associated with increased mortality and an increased risk of CV disease, which is not explained by traditional CV risk factors alone^{4,11,12}. This increased risk is likely due to disease-related factors, including both systemic inflammation and immune dysregulation. Importantly, both systemic inflammation and immune dysregulation, including autoantibody production, may occur even in the absence of autoimmune rheumatic diseases, and these factors could contribute to the pathogenesis of atherosclerosis^{13,14}. In our study, we show that immune dysregulation, as manifested by the presence of the commonly tested autoantibodies RF and ANA, is associated with CV events and overall mortality both in those with and those without rheumatic diseases.

Inflammation [as studied through elevations in C-reactive protein (CRP) and/or erythrocyte sedimentation rate] contributes to increased risk of CVD, both in patients with autoimmune diseases and in the general population^{1,2,5,15,16}. Besides systemic inflammation, another hallmark of

autoimmune diseases is immune dysregulation, manifested by the presence of autoantibodies, which may also be important in mediating CV risk. Indeed, RF positivity has been found to predict mortality in several studies of patients with RA^{7,17}. Similarly, CCP, an increasingly utilized autoantibody test, has been found to predict radiographic progression and mortality in RA^{18,19}. Hence, CCP appears to be a marker, like RF, that portends a more aggressive disease course, along with higher degrees of systemic inflammation. However, unlike RF, overall there have been generally few population-based studies assessing the risk of CV morbidity and mortality from CCP.

It is also well established that patients with SLE are at increased risk of premature coronary atherosclerosis^{4,20}. Perhaps more than any other autoimmune disease, a hallmark of SLE is the presence of autoantibodies, in particular ANA. In one study, high titers of serum ANA, mostly directed against nucleolar antigens, were associated with atherosclerosis in patients without any autoimmune disorder²¹. However, again, overall there are few studies, either epi-

demologic or pathophysiologic, examining the possible role of ANA in predicting or mediating atherogenesis.

The vast amount of research on CVD risk in the rheumatic diseases, and in particular, the increased risk of CV outcomes in seropositive patients with RA, has potential implications for understanding the pathogenesis of atherosclerosis. Specifically, if the presence of autoantibodies such as RF and ANA contributes to the increased CVD risk in patients with rheumatic diseases, perhaps they also contribute to increased CVD risk in those without clinically evident rheumatic diseases. So far, few studies have addressed this important research question. In a cross-sectional study in England, RF was found to be associated with a 3-fold increased risk of ischemic events in men²². Yet there was no significant association between RF and ischemic disease in women, or between ANA or anticardiolipin antibody and ischemic disease in men or women. In a case-control study from eastern Finland, RF and ANA, as determined from baseline specimens, were shown to predict cardiovascular mortality, but the effect was mainly confined to subjects who were seropositive for RF, as there were very small numbers of subjects with positive ANA tests²³. In another longitudinal population-based Finnish study, patients without arthritis with “false-positive RF” titers of ≥ 128 were found to have a 74% increased risk of CV deaths²⁴. Further, in RA and Felty’s syndrome, RF was shown to augment immunoglobulin binding to endothelial cells *in vitro*, and antiendothelial cell antibodies were detected in RA, Felty’s syndrome, SLE, and lupus anticoagulant sera²⁵. In other *in vitro* studies, additional autoantibodies have also been implicated in endothelial cell dysfunction, which is one of the first steps in atherogenesis; we have recently reviewed this literature²⁶.

Despite these studies, the potential role of autoantibodies in predicting CV risk and mortality in those without rheumatic disease remains unclear. First, the results were conflicting regarding ANA positivity, with one showing no increase in ischemic heart disease in ANA-positive men or women²², and the other suggesting an increase in CV death²³. Second, it was not clear whether the observed associations were due to the presence of rheumatic disease in those tested positive for autoantibodies. Third, there were few data on the risk of specific CV events such as MI, HF, or PVD. Finally, there was little evidence for gender differences between the risk of heart disease in those with RF positivity, with only one study showing that RF positivity predicted CVD in men but not women²².

Our study extends these earlier observations, by demonstrating that both RF and ANA positivity are predictive of CV events and mortality both in those with and those without rheumatic diseases. This finding lends further support to the hypothesis of immune dysregulation playing an important role in atherosclerosis even in those without rheumatic diseases. In addition, these findings were consistent in both

men and women. Further, our study is the first to demonstrate a “dose effect” where both “strong positive” RF and ANA had a greater effect on risk of CV outcomes than “weak positive” RF and ANA. In addition, while we cannot entirely eliminate the possibility of confounding by indication of autoantibody testing, our findings are unlikely to be the result of it, as there was no significant difference in risk of CV outcomes in patients tested for autoantibodies compared to patients not tested for them.

Potential limitations of our study include lack of data on CRP values that may potentially act as an effect modifier, short followup time for CCP testing and small numbers of CCP-positive individuals, limited generalizability to different populations, possible incomplete assessment of confounding by indication since chronic disease comorbidities do not include acute conditions that may potentially be associated with autoantibody positivity, and lack of validation of rheumatic and CV disease diagnoses using established classification criteria. Nevertheless, the potential for misclassification would be expected to be similar in both those with and without autoantibody testing, as well as those positive versus negative for autoantibody tests. Even if there was systematic bias in misclassifying those with positive autoantibody tests as having rheumatic diseases, analyses adjusting for the occurrence of rheumatic disease would be biased toward the null. While our findings were statistically significant, the estimated risks of RF and ANA positivity on CV outcomes were modest (HR < 2), indicating that RF and ANA contribute only modestly to CV risk. Finally, another limitation was that our study included RF and ANA testing by different methods. However, the enzyme immunoassay and indirect immunofluorescence methods of ANA testing performed at our institution have been comprehensively evaluated in the past and found to be essentially equivalent²⁷.

Our study also has several strengths. It is the first to investigate the predictive value of CCP positivity on CV events and mortality, and one of the few to investigate the predictive value of RF and ANA positivity on CV events and mortality in subjects without clinically evident rheumatic diseases. Our study also investigated the possibility that the relationship between autoantibody testing and development of CV events and mortality may be affected by confounding by indication, which lends greater validity to the results. Mean followup was long (~ 9 yrs for RF and ANA) and complete for all autoantibody testing and clinically recognized CV outcomes and rheumatic diseases.

Our results support an important role of the autoantibodies RF and ANA in mediating cardiovascular disease in both individuals with and those without clinically evident rheumatic diseases, and provide hypothesis-generating insights into potential pathogenetic mechanisms of atherosclerosis. The presence of autoantibodies as a marker of immune dysregulation, while traditionally thought of as “false-positive”

clinically, may have potential implications for future CV risk. Further investigations regarding the role of autoantibodies in the pathogenesis of atherosclerosis and cardiovascular disease are needed.

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APPENDIX. International Classification of Disease 9 (ICD-9) codes of cardiovascular outcomes, rheumatic diseases, and comorbidities.

Diagnoses	ICD-9 Codes
<i>Cardiovascular outcomes</i>	
Myocardial infarction	410.x, 412.x
Heart failure	428.x, 402.x
Peripheral vascular disease	440.0-443.9, 785.4, V43.4
<i>Rheumatic diseases</i>	
Rheumatoid arthritis	714.0-714.2, 714.81
Polymyalgia rheumatica	725.0
Systemic lupus erythematosus	710.0
Other connective tissue diseases	710.1, 710.4
<i>Comorbidities</i>	
<i>Infections</i>	
Tuberculosis, leprosy, mycobacterial, streptococcal/meningitis, sepsis/septicemia, Varicella/measles/Rubella, hepatitis, mumps, Coxsackie/mononucleosis/EBV, viral illness/disease/infection, malaria/syphilis, venereal disease/leptospirosis, schistosomiasis, rheumatic heart disease, endocarditis, influenza, erythema nodosum/multiforme/annulare, AIDS/HIV+	010-018.x, 137.0-4, 030-031.x, 034.x, 036-038.x, 041.85, 050-057.x, 139.8, 771.0, 771.2, 070.x, 790.99, 072.8-9, 074.x-075, 078.x-079, 084.x-100.x, 104.0, 120.x-130.x, 391.x-394.x, 421.x, 424.x, 487.1, 487.8, 695.0-2, 279.1-2, 042.9, 043.9, 785.6, 795.71, 795.8
<i>Nonrheumatic autoimmune diseases</i>	
Hashimoto's thyroiditis	245.2, 245.8
Graves' disease	242.0, 242.8, 376.2x, 359.5
Autoimmune hepatitis	573.3
Primary biliary cirrhosis	571.6
Primary autoimmune cholangitis	576.1
Pulmonary arterial hypertension	401.9, 416.0
Sarcoidosis	135, 425.8
Interstitial pulmonary fibrosis	515.x, 516.x, 136.3
Silicosis	502
Asbestosis	501, 989.81
<i>Chronic disease comorbidities*</i>	
Diabetes (with or without acute metabolic disturbances; with peripheral circulatory disorders)	250-250.3, 250.7
Diabetes with chronic complications (renal, ophthalmic, or neurological manifestations)	250.4-250.6
Cerebrovascular disease	430-438
Dementia (senile and presenile)	290-290.9
Chronic pulmonary disease (COPD, pneumoconioses, chronic respiratory conditions due to fumes and vapors)	490-496, 500-505, 506.4
Peptic ulcer disease (gastric, duodenal and gastrojejunal ulcers; chronic forms of PUD)	531-534.9, 531.4-531.7, 532.4-532.7, 533.4-533.7, 534.4-534.7
Mild liver disease (alcoholic cirrhosis,	571.2, 571.5, 571.6, 571.4-

cirrhosis without mention of alcohol,	571.49
biliary cirrhosis, chronic hepatitis)	
Moderate or severe liver disease (hepatic coma, portal HTN, other sequelae; esophageal varices)	572.2-572.8, 456.0-456.21
Hemiplegia or paraplegia	342-342.9, 344.1
Renal disease (chronic GN; nephritis and nephropathy; chronic renal failure; renal failure, unspecified; disorders resulting from impaired renal function)	582-582.9, 583-583.7, 585, 586, 588-588.9
Any malignancy, including leukemia and lymphoma (excluding skin cancer other than melanoma)	140-172.9, 174-195.8, 200-208.9
Metastatic solid tumor (secondary malignant neoplasm of lymph nodes and other organs)	196-199.1
AIDS (HIV infection with related specified conditions)	042-044.9

EBV: Epstein-Barr virus; AIDS: acquired immunodeficiency syndrome; HIV: human immunodeficiency virus; COPD: chronic obstructive pulmonary disease; PUD: peptic ulcer disease; GN: glomerulonephritis; HTN: hypertension. *Adapted from Charlson comorbidity index components^{9,10}.

REFERENCES

- Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;336:973-9.
- Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation* 1998;98:731-3.
- Gabriel SE, Crowson CS, Kremers HM, Doran MF, Turesson C, O'Fallon WM, et al. Survival in rheumatoid arthritis: a population-based analysis of trends over 40 years. *Arthritis Rheum* 2003;48:54-8.
- Doria A, Iaccarino L, Sarzi-Puttini P, Atzeni F, Turriel M, Petri M. Cardiac involvement in systemic lupus erythematosus. *Lupus* 2005;14:683-6.
- Maradit-Kremers H, Nicola PJ, Crowson CS, Ballman KV, Jacobsen SJ, Roger VL, et al. Raised erythrocyte sedimentation rate signals heart failure in patients with rheumatoid arthritis. *Ann Rheum Dis* 2007;66:76-80.
- Maradit-Kremers H, Nicola PJ, Crowson CS, Ballman KV, Gabriel SE. Cardiovascular death in rheumatoid arthritis: a population-based study. *Arthritis Rheum* 2005;52:722-32.
- Goodson NJ, Wiles NJ, Lunt M, Barrett EM, Silman AJ, Symmons DP. Mortality in early inflammatory polyarthritis: cardiovascular mortality is increased in seropositive patients. *Arthritis Rheum* 2002;46:2010-9.
- Melton LJ 3rd. History of the Rochester Epidemiology Project. *Mayo Clin Proc* 1996;71:266-74.
- Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987;40:373-83.
- Deyo RA, Cherkin DC, Ciol MA. Adapting a clinical comorbidity index for use with ICD-9-CM administrative databases. *J Clin Epidemiol* 1992;45:613-9.
- Nicola PJ, Crowson CS, Maradit-Kremers H, Ballman KV, Roger VL, Jacobsen SJ, et al. Contribution of congestive heart failure and ischemic heart disease to excess mortality in rheumatoid arthritis. *Arthritis Rheum* 2006;54:60-7.
- Maradit-Kremers H, Crowson CS, Nicola PJ, Ballman KV, Roger VL, Jacobsen SJ, et al. Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a

- population-based cohort study. *Arthritis Rheum* 2005;52:402-11.
13. Poston RN, Davies DF. Immunity and inflammation in the pathogenesis of atherosclerosis. A review. *Atherosclerosis* 1974;19:353-67.
 14. Binder CJ, Chang MK, Shaw PX, Miller YI, Hartvigsen K, Dewan A, et al. Innate and acquired immunity in atherogenesis. *Nat Med* 2002;8:1218-26.
 15. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med* 2004;350:1387-97.
 16. Goodson NJ, Symmons DP, Scott DG, Bunn D, Lunt M, Silman AJ. Baseline levels of C-reactive protein and prediction of death from cardiovascular disease in patients with inflammatory polyarthritis: a ten-year followup study of a primary care-based inception cohort. *Arthritis Rheum* 2005;52:2293-9.
 17. Wolfe F, Mitchell DM, Sibley JT, Fries JF, Bloch DA, Williams CA, et al. The mortality of rheumatoid arthritis. *Arthritis Rheum* 1994;37:481-94.
 18. Meyer O, Nicaise-Roland P, Santos MD, Labarre C, Dougados M, Goupille P, et al. Serial determination of cyclic citrullinated peptide autoantibodies predicted five-year radiological outcomes in a prospective cohort of patients with early rheumatoid arthritis. *Arthritis Res Ther* 2006;8:R40.
 19. Farragher TM, Goodson NJ, Naseem H, Silman AJ, Thomson W, Symmons D, et al. Association of the HLA-DRB1 gene with premature death, particularly from cardiovascular disease, in patients with rheumatoid arthritis and inflammatory polyarthritis. *Arthritis Rheum* 2008;58:359-69.
 20. Manzi S, Meilahn EN, Rairie JE, Conte CG, Medsger TA Jr, Jansen-McWilliams L, et al. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. *Am J Epidemiol* 1997;145:408-15.
 21. Grainger DJ, Bethell HW. High titres of serum antinuclear antibodies, mostly directed against nucleolar antigens, are associated with the presence of coronary atherosclerosis. *Ann Rheum Dis* 2002;61:110-4.
 22. Edwards CJ, Syddall H, Goswami R, Goswami P, Dennison EM, Arden NK, et al; Hertfordshire Cohort Study Group. The autoantibody rheumatoid factor may be an independent risk factor for ischaemic heart disease in men. *Heart* 2007;93:1263-7.
 23. Aho K, Salonen JT, Puska P. Autoantibodies predicting death due to cardiovascular disease. *Cardiology* 1982;69:125-9.
 24. Heliövaara M, Aho K, Knekt P, Aromaa A, Maatela J, Reunanen A. Rheumatoid factor, chronic arthritis and mortality. *Ann Rheum Dis* 1995;54:811-4.
 25. Quadros NP, Roberts-Thomson PJ, Gallus AS. IgG and IgM anti-endothelial cell antibodies in patients with collagen-vascular disorders. *Rheumatol Int* 1990;10:113-9.
 26. Liang KP, Gabriel SE. Autoantibodies: innocent bystander or key player in immunosenescence and atherosclerosis? *J Rheumatol* 2007;34:1203-7.
 27. Homburger HA, Cahen YD, Griffiths J, Jacob GL. Detection of antinuclear antibodies: comparative evaluation of enzyme immunoassay and indirect immunofluorescence methods. *Arch Pathol Lab Med* 1998;122:993-9.