RHEUMATOLOGY

Rheumatology 2015;54:1587–1595
doi:10.1093/rheumatology/kev027
Advance Access publication 7 April 2015

Original article

Vascular calcifications on hand radiographs in rheumatoid arthritis and associations with autoantibodies, cardiovascular risk factors and mortality

E. Blair Solow[1], Fang Yu[2], Geoffrey M. Thiele[3], Jeremy Sokolove[4], William H. Robinson[4], Zachary M. Pruhs[3], Kaleb D. Michaud[3,5], Alan R. Erickson[3], Harlan Sayles[3], Gail S. Kerr[6], Angelo L. Gaffo[7], Liron Caplan[8], Lisa A. Davis[8], Grant W. Cannon[9], Andreas M. Reimold[1,10], Joshua Baker[11], Pascale Schwab[12], Daniel R. Anderson[13] and Ted R. Mikuls[3]

Abstract

Objective. To examine whether vascular calcifications on hand films in RA might aid in determining mortality risk.

Methods. Hand radiographs from 906 RA patients were scored as positive or negative for vascular calcifications. Patient characteristics associated with vascular calcifications were assessed using multivariable logistic regression, and associations with mortality were examined using Cox proportional hazards regression. Cytokines and multiplex ACPA were measured in both groups.

Results. A total of 99 patients (11%) demonstrated radiographic vascular calcifications. Factors independently associated with vascular calcifications included diabetes [odds ratio (OR) 2.85; 95% CI 1.43, 5.66], cardiovascular disease at enrolment (OR 2.48; 95% CI 1.01, 6.09), prednisone use (OR 1.90; 95% CI 1.25, 2.91), current smoking (OR 0.06; 95% CI 0.01, 0.23) and former smoking (OR 0.36; 95% CI 0.27, 0.48) *vs* never smoking. In cytokine and ACPA subtype analysis, IL-4 and anti-citrullinated apolipoprotein E were significantly increased in patients with vascular calcifications in fully adjusted multivariable models. After multivariable adjustment, vascular calcifications were associated with an increase in all-cause mortality (hazard ratio 1.41; 95% CI 1.12, 1.78; $P = 0.004$).

Conclusion. Vascular calcifications on hand radiographs were independently associated with increased all-cause mortality in RA. Mechanisms underpinning the associations of IL-4 and select ACPA with vascular calcifications and their utility as biomarkers predictive of cardiovascular disease risk in RA merit further study.

Key words: rheumatoid arthritis, vascular calcifications, hand radiographs, anti-citrullinated protein autoantibodies, mortality.

[1]Division of Rheumatic Diseases, UT Southwestern, Dallas, TX, [2]Department of Biostatistics, College of Public Health, University of Nebraska Medical Center, Omaha, [3]Division of Rheumatology and Immunology, Omaha Veterans Affairs Medical Center, Omaha, NE, [4]Division of Rheumatology, VA Palo Alto Health Care System, Palo Alto, CA, [5]National Data Bank for Rheumatic Diseases, Wichita, KS, [6]Department of Medicine, Veterans Affairs Medical Center, Washington, DC, [7]Department of Medicine, Birmingham Veterans Affairs Medical Center, Birmingham, AL, [8]Division of Rheumatology, Denver Veterans Affairs Medical Center, Denver, CO, [9]Division of Rheumatology, George Wahlen Veterans Affairs Medical Center, Salt Lake City, UT, [10]Division of Rheumatology, Dallas Veterans Affairs Medical Center, Dallas, TX, [11]Division of Rheumatology, Philadelphia Veterans Affairs Medical Center, Philadelphia, PA, [12]Division of Rheumatology, Portland Veterans Affairs Medical Center, Portland, OR and [13]Division of Cardiology, University of Nebraska Medical Center, Omaha, NE

Submitted 15 October 2014; revised version accepted 9 February 2015

Correspondence to: Ted R. Mikuls, Division of Rheumatology and Immunology, Omaha Veterans Affairs Medical Center, 986270 Nebraska Medical Center, Omaha, NE 68198-6270, USA.
E-mail: tmikuls@unmc.edu

Rheumatology key messages

- Radiographic vascular calcifications may be informative in a comprehensive cardiovascular disease risk assessment in RA.
- In RA auto-antibodies to particular citrullinated antigens may contribute to vascular pathology.

Introduction

Patients with RA are at a substantially increased risk of morbidity and mortality related to cardiovascular disease (CVD) [1, 2]. However, investigations examining the association of radiographically detected vascular calcifications with CVD in this high-risk patient population are limited. Previous studies have demonstrated a relationship between radiographic vascular calcifications and mortality in other populations. For instance, in patients with non–insulin-dependent diabetes mellitus, the presence of arterial calcifications on plain radiography has been associated with a >4-fold increase in mortality [odds ratio (OR) 4.2; 95% CI 1.3, 11.3] [3]. Among patients with end-stage renal disease, radiographic arterial calcifications have been associated with reduced survival [4]. The magnitude of coronary artery calcifications, quantified using CT, has increasingly been used to screen and stratify individuals for CVD risk [5]. Several preliminary studies, relying predominantly on the use of US or CT measurement, have demonstrated increased prevalence and magnitude of arterial calcifications in the coronary and carotid arteries and the thoracic aorta in RA patients compared with non-RA controls [6–8]. These studies have been cross-sectional in design, limiting inferences that can be made regarding the directional nature of these relationships.

In RA, there has been no study to date that has prospectively examined the prognostic implications of radiographically detected vascular calcifications and occurrence of CVD events or mortality. At present, clinicians have few tools available to accurately stratify CVD and related mortality risk in the context of RA care. Risk assessment tools that rely solely on conventional risk factors, such as hypertension and hyperlipidaemia, do not adequately explain the excess burden of atherosclerosis observed in RA [9]. Although imaging techniques such as carotid artery US and coronary CT may have the potential of yielding high predictive power for future cardiac events [5, 10], these modalities are not routinely employed clinically. In contrast, RA patients routinely undergo radiographic assessments for diagnosis and to gauge arthritis progression and treatment response. Whether hand radiographs commonly obtained as part of RA care might prove to be informative in the CVD risk stratification strategy is unknown.

In the present study, we sought to determine whether commonly performed radiographs, namely X-ray studies of the hands, might provide valuable information relative to CVD risk assessment in RA. We investigated the frequency of vascular calcifications found on hand radiographs and the clinical factors associated with their presence. In the light of recent reports demonstrating the presence of citrullinated autoantigens in atherosclerotic lesions and the associations of disease-related autoantibody with CVD risk [11], we explored the association of antigen-specific ACPA with the presence of vascular calcifications. We subsequently examined the relationship between vascular calcifications and all-cause mortality.

Patients and methods

Study population

Patients were participants in the Veterans Affairs Rheumatoid Arthritis (VARA) registry, a longitudinal observational study of US veterans with RA [12, 13]. In addition to serving as a biological repository, the registry includes digitized hand radiographs collected as part of routine care and obtained at the discretion of the treating rheumatologist. Initiated in 2003, VARA is a multicentre effort that currently involves active collection sites at 10 VA Medical Centers across the USA (Birmingham, AL; Dallas, TX; Denver, CO; Iowa City, IA; Jackson, MS; Omaha, NE; Philadelphia, PA; Portland, OR; Salt Lake City, UT; and Washington, DC). Participants satisfy the 1987 ACR classification criteria for RA [14] and have a disease onset after 18 years of age. The registry has received Institutional Review Board approval at each site, and all study subjects provide written informed consent before enrolment. This study was also approved by the VARA Scientific Ethics Advisory Committee.

Hand radiographs

Digitized hand radiographs, which included views of the wrists, were examined using a sample of 906 RA participants with at least one radiographic study per patient. For patients with multiple films, we examined the earliest study completed demonstrating vascular calcifications. If no vascular calcifications were found on any of the available studies, the last set of radiographs was used. The mean lag time between the date of study enrolment and radiographic examination was 2.7 months (median 0 months). Radiographs were read in a blinded fashion by investigators (Z.P. and A.E.) and scored as either positive or negative for the presence of vascular calcifications. Inter-rater agreement was assessed using 533 films read by both investigators. The two readers exhibited 92% concordance; however, the kappa coefficient reflected moderate agreement ($\kappa = 0.49$) owing to one reader classifying more films as positive for vascular calcifications.

Clinical variables

Patient characteristics examined included demographic and health behaviours, comorbidities and RA-related factors. Demographic- and health-related measures examined included age, race, gender, education ($\geqslant$high school *vs* $<$high school), smoking status at enrolment

(never, former, current) and BMI (kg/m^2). BMI was examined as a continuous variable with and without a spline knot at 23 kg/m^2 as a threshold, the latter based on prior work highlighting the relevance of this threshold to mortality risk [15]. Comorbid conditions were defined using International Classification of Disease-9-clinical modification codes documented at the time of registry enrolment and included diabetes, hypertension, hyperlipidaemia, chronic kidney disease, CVD and cerebrovascular disease, each examined as a dichotomous variable, see supplementary Table S1, available at *Rheumatology* Online.

RA-related factors examined included RA disease duration (years), ACPA and RF positivity, Routine Assessment of Patient Index Data-3 (RAPID-3) [16, 17] and DAS-28 using ESR scores [18], CRP concentration, the presence of rheumatoid nodules, usage of prednisone, MTX or biologics (adalimumab, etanercept, infliximab, rituximab or abatacept) at enrolment and throughout follow-up, and lastly, cytokine profiles and antibodies targeting RA-associated citrullinated antigens (detailed below).

Autoantibody and cytokine assays

ACPA was measured using a second generation anti-CCP antibody ELISA (Diastat, Axis-Shield Diagnostics Ltd, Dundee, UK; positive if $\geqslant$5 U/ml), while RF (positive if $\geqslant$15 IU/ml) and high-sensitivity CRP concentrations were determined by nephelometry (Siemens Healthcare Diagnostics, Munich, Germany). Autoantibodies targeting 17 putative RA-associated autoantigens were measured using a custom bead-based immunoassay on a Bio-Plex platform [11, 19]. Briefly, serum was diluted and mixed with spectrally distinct fluorescent beads conjugated with putative RA-associated autoantigens, followed by incubation with anti-human phycoerythrin-labelled antibody, and analysed on a Luminex 200 instrument.

Multiplex analysis of cytokines and chemokines was performed using a Bio-Plex Pro Human Cytokines 17-plex Assay (Bio-Rad, Hercules, CA, USA) run on a Luminex 200 system according the manufacturer's instructions, with the exception that a proprietary Bio-Rad assay dilution buffer was modified to contain reagents demonstrated to reduce the effects of heterophilic antibodies in multiplex immunoassays [19]. Data processing was performed using Bio-Plex Manager 5.0 software, and analyte concentrations (picograms per millilitre) were interpolated from standard curves. All analyses were performed using banked serum from enrolment.

Statistical analysis

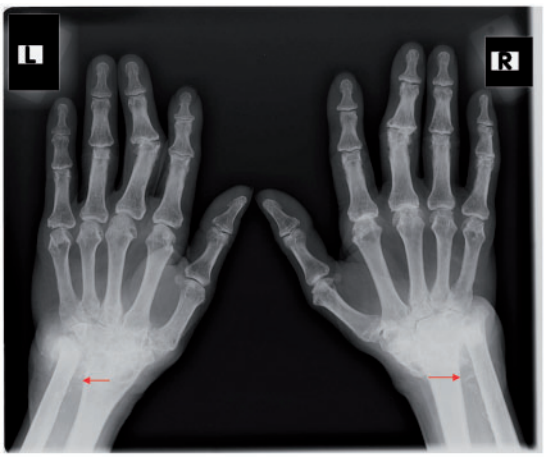
Patients with and without evidence of radiographically detected vascular calcifications were compared with regard to demographic characteristics and the presence of CVD risk factors present at enrolment, using the *t*-test for continuous variables and the chi-squared test for categorical variables. The association of patient characteristics and RA-related disease features with the presence of radiographic vascular calcifications was subsequently examined using backwards stepwise multivariable logistic regression. All variables retained in the final model had a $P < 0.05$. Additional comparisons were performed utilizing multiplex cytokines and antigen-specific ACPA array data by Significance Analysis of Microarrays (SAM) version 3.08. In the initial SAM screen, output was sorted based on false discovery rates to account for multiple comparisons. Subsequently, we identified the antigens with the greatest differences in autoantibody reactivity between vascular calcification subgroups [19]. Median (quantile) regression was performed to examine the independent associations of cytokines/chemokines and ACPA, identified via initial SAM screening, with the presence of vascular calcifications. Using this approach, median values of cytokines/chemokines and ACPAs were modelled as independent variables rather than the mean values to reduce the influence of outliers. Four multivariate models were assembled and included the following covariates: (i) age and gender; (ii) model 1 plus RA disease duration, RAPID-3 and prednisone use; (iii) model 2 plus hypertension, hyperlipidaemia, diabetes, BMI, CRP and smoking status; and (iv) model 3 plus prevalent CVD at enrolment.

The association of radiographic vascular calcifications with all-cause mortality was examined using Cox proportional hazards regression with initial age adjustment and stratified by gender, followed by multivariable adjustments. RA-related measures, including measures of disease activity and medications, were treated as time-varying with the exception of ACPA, RF and CRP, which were measured at a single time point using baseline serum. Mortality events were captured using annual abstraction of the VA computerized patient record system, by next-of-kin notification or by periodic regional VA surveillance as previously detailed [12]. Thirty-eight subjects were excluded from this analysis as they did not meet the criteria of having two observations or a date of death needed to define a follow-up interval. The at-risk period extended from the time of enrolment until death or the last available registry observation. With the exception of the presence of vascular calcifications, which was forced into all models, only variables with a $P < 0.1$ in age-adjusted (gender-stratified) analyses were entered into the initial multivariable model. The final model was then selected by step-wise removal until all remaining *P*-values were <0.05. To further explore the association of vascular calcifications and mortality in subjects with diabetes, we estimated two separate models, treating those with and without diabetes as separate samples. We then performed univariate analysis separately on our two subsamples, followed by backwards stepwise selection with the two different variable lists that came from the univariate analysis. Cause-specific mortality data were not available for this analysis. All analyses were completed using Stata version 12 (StataCorp, College Station TX, USA).

Results

Vascular calcifications were observed in hand radiographs in 99 (11%) of the 906 patients studied (Fig. 1). Patient characteristics for those with and without vascular calcifications are shown in Table 1. Patients with vascular calcifications were older, more often male and had lower

FIG. 1 Vascular calcifications (arrows) on a plain radiograph in a patient with RA



BMI at enrolment. Patients with vascular calcifications more often had associated comorbidities, including diabetes mellitus, chronic renal disease and CVD, had longer RA disease duration, higher RAPID-3 scores and a higher rate of prednisone use compared with patients without vascular calcifications. The majority of patients with vascular calcifications were seropositive for ACPA and RF, although these rates were not substantially different from those without vascular calcifications. Notably, ever smoking was significantly less common among those with vascular calcifications than those without (62% *vs* 82%; $P < 0.001$).

In multivariable analysis, factors independently associated with vascular calcifications included diabetes mellitus (OR 2.85; 95% CI 1.43, 5.66; $P = 0.003$), prevalent CVD (OR 2.48; 95% CI 1.01, 6.09; $P < 0.047$) and prednisone usage (OR 1.9; 95% CI 1.25, 2.91; $P = 0.003$) (Table 2). Compared with never smokers, both former (OR 0.36; 95% CI 0.27, 0.48; $P < 0.001$) and current smokers (OR 0.06; 95% CI 0.01, 0.23; $P < 0.001$) were significantly less likely to have vascular calcifications following multivariable adjustment.

Although ACPA status was not associated with vascular calcifications, we examined whether ACPA subspecificities and cytokines/chemokines were overrepresented in RA subjects with vascular calcifications. Associations were analysed using SAM analysis and the output sorted by false discovery rates. Using SAM screening, IL-4, TNFα and ACPA targeting citrullinated forms of apolipoprotein E (anti-Cit-ApoE), vimentin (anti-Cit-vim) and fibrinogen (anti-Cit-fib) were found to be significantly elevated in subjects with vascular calcifications. In multivariable analyses, associations of anti-Cit-ApoE and IL-4 with the presence of vascular calcifications remained significant in the fully adjusted model (model 4), and only the association of anti-Cit-ApoE with vascular calcifications remained significant in all models (Table 3).

To determine the mortality risk associated with vascular calcifications, we performed age-adjusted and gender-stratified Cox proportional hazard regression (Table 4). Of the 868 patients included in the all-cause mortality analysis, there were 179 deaths (20.6%) during a mean follow-up period of 5.0 (S.D. 3.5) years. Vascular calcifications were associated with a significantly higher all-cause mortality after adjustment for age and gender, and remained significant after multivariable adjustment [hazard ratio (HR) 1.41; 95% CI 1.12, 1.78; $P < 0.004$]. Other variables that remained significantly associated with increased mortality risk in the multivariate model included age, smoking, low BMI, chronic kidney disease, CVD, RAPID-3 score and CRP. In age- and gender-adjusted models, log-transformed anti-Cit-ApoE values (but not anti-Cit-vim or anti-Cit-fib) were associated with all-cause mortality (HR 1.16; 95% CI 1.08, 1.24). This association was attenuated and non-significant in multivariable models (data not shown). MTX and biologic use were associated with a significantly lower HR for all-cause mortality. To investigate the role of diabetes, we performed Cox proportional hazard regression in subjects with and without diabetes. We found vascular calcifications remained significantly associated with all-cause mortality in both groups (data not shown).

Discussion

To our knowledge, this is the first report to define both the frequency and clinical correlates of vascular calcifications using plain radiographs of the hands and wrists from patients with RA. Likewise, this is the first investigation to evaluate all-cause mortality risk in RA associated with vascular calcifications. Our major finding was that patients with vascular calcifications detected in the context of routine rheumatology care demonstrated a greater all-cause mortality risk that was independent of factors that have been linked to an increased risk of cardiovascular events, including comorbid chronic kidney disease and CVD.

CVD has previously been considered a disease of lipid deposition. There is now a greater understanding of the complex inflammatory processes driving atherosclerosis. Emerging evidence points to the striking similarities in the inflammatory pathways that characterize both atherosclerosis and RA. Libby and colleagues [20] reported on a large body of evidence linking atherosclerosis to the sequelae of systemic inflammation, including the impact of the increased expression of pro-inflammatory cytokines such as IL-6 and TNFα that characterize RA. Further, by alleviating inflammation, the risk of developing CVD in RA may be mitigated [21, 22].

Our study demonstrates increased circulating levels of the inflammatory cytokines IL-4 and TNFα in subjects with vascular calcifications, although only the association with IL-4 remained significant after full multivariate adjustment. In human studies, TNFα is associated with coronary calcium burden in RA [23], and biologic agents targeting TNFα have been found to reduce cardiovascular events [21, 24]. The role of IL-4 in the pathogenesis of atherosclerosis is controversial. IL-4 may be protective against

Table 1 Clinical characteristics and RA-related factors in patients at enrolment

Variable	Vascular calcifications present ($n = 99$)	Vascular calcifications absent ($n = 807$)	*P*-value
Demographics and health behaviours			
Age, mean (S.D.), years	72 (9)	64 (11)	<0.001
Men, *n* (%)	96 (97)	725 (90)	0.02
⩾High school education, *n* (%)	75 (82)	654 (86)	0.40
Race, *n* (%)			
Caucasian	79 (80)	615 (76)	0.53
African American	13 (13)	142 (18)	
Other	7 (7)	50 (6)	
Smoking, *n* (%)			
Never	38 (38)	146 (18)	<0.001
Former	53 (54)	427 (53)	
Current	8 (8)	234 (29)	
BMI, mean (S.D.), kg/m^2	27 (5)	28 (5)	0.02
Comorbidity, *n* (%)			
Diabetes	38 (38)	139 (17)	<0.001
Hypertension	67 (68)	464 (58)	0.05
Hyperlipidaemia	45 (45)	343 (43)	0.58
Chronic kidney disease	12 (12)	43 (5)	0.008
Cardiovascular disease	43 (43)	164 (20)	<0.001
Cerebrovascular disease	7 (7)	57 (7)	0.99
RA-related factors			
Disease duration, mean (S.D.), years	16 (13)	13 (11)	0.004
ACPA-positive, *n* (%)	74 (77)	620 (78)	0.79
RF-positive, *n* (%)	73 (75)	635 (80)	0.26
RAPID-3 score, mean (S.D.)	2.9 (1.3)	2.5 (1.4)	0.02
DAS-28 score, mean (S.D.)	3.9 (1.5)	3.8 (1.5)	0.29
CRP, mean (S.D.), mg/l	14 (24)	11 (18)	0.21
Nodules, *n* (%)	44 (44)	337 (42)	0.61
MTX use, *n* (%)	51 (53)	427 (56)	0.52
Prednisone use, *n* (%)	53 (55)	313 (41)	0.01
Biologic use, *n* (%)	33 (33)	262 (32)	0.86

Biologics included anti-TNF agents (adalimumab, etanercept and infliximab), abatacept and rituximab. RAPID: routine assessment of patient index data.

Table 2 Multivariable associations of baseline patient characteristics with the presence of vascular calcifications on plain radiographs of the hands and wrists

Variable	Odds ratio (95% CI)	*P*-value
Diabetes	2.85 (1.43, 5.66)	0.003
Cardiovascular disease	2.48 (1.01, 6.09)	0.047
Prednisone	1.90 (1.25, 2.91)	0.003
Smoking		
Never	Referent	
Former	0.36 (0.27, 0.48)	<0.001
Current	0.06 (0.01, 0.23)	<0.001

developing stenotic arterial lesions and is associated with reduced CVD risk in the general population [25, 26]. However, animal models of atherosclerosis suggest IL-4 contributes to the late stages of plaque development [27]. In osteoimmunology models, IL-4 has been shown to decrease osteoprotegrin levels and promote an osteoblastic phenotype from coronary artery smooth muscle cells leading to increased mineralization *in vitro* [28]. Mice overexpressing IL-4 develop osteoporosis [29]. Mice deficient in osteoprotegrin are not only osteoporotic but develop vascular calcifications [30]. Further studies are required to determine whether IL-4 may be involved in calcific vasculopathies, particularly in RA patients.

We identified an increased concentration of circulating ACPA targeting citrullinated forms of fibrinogen, vimentin and ApoE in RA patients with vascular calcifications. Of these, only the association of anti-Cit-ApoE antibody with vascular calcifications remained significant across all multivariable models. ApoE is well recognized to play a central role in lipoprotein metabolism in addition to exerting anti-inflammatory effects. Defects in ApoE2 result in familial dysbetalipoproteinaemia, suggesting that ApoE affords protection against the development of atherosclerosis [31]. Further, genetic variants of ApoE, such as

Table 3 Multivariable quantile regression[a] of ACPA subtypes and cytokines and the associations with radiographic vascular calcifications

Variable ACPA or cytokine/ chemokine	Significance analysis of microarray		Multivariable quantile regression, *P*-value			
	Fold change VC^+ *vs* VC^-	*Q*-value, %	Model 1	Model 2	Model 3	Model 4
ACPA subtype						
Anti-citrullinated ApoE	1.28	0	0.013	0.003	0.018	0.034
Anti-citrullinated vimentin	1.35	0	0.021	0.025	0.084	0.121
Anti-citrullinated fibrinogen	1.30	0	0.487	0.496	0.601	0.601
Cytokine/chemokine						
IL-4	1.37	0	0.063	0.117	0.019	0.021
TNF-α	1.26	0	0.456	0.335	0.045	0.101

ACPA subtypes and cytokines generated by significance analysis of microarrays analysis followed by multivariable quantile regression.[a]Models include adjustments for: age and gender; model 1 plus RA-related measures, including disease duration, RAPID-3 and prednisone use; model 2 plus traditional CVD risk factors, including hypertension, hyperlipidaemia, diabetes, BMI, CRP and smoking status (current, former *vs* never); and model 3 plus prevalent CVD at the time of enrolment. VC: vascular calcification.

Table 4 Hazard ratios for all-cause mortality from age-adjusted and gender-stratified univariate and multivariable analysis

Variable	Age-adjusted and gender-stratified univariate HR (95% CI)	*P*-value	Multivariable HR (95% CI)	*P*-value
Vascular calcification	1.36 (1.06, 1.74)	0.02	1.41 (1.12, 1.78)	0.004
Demographics and health behaviours				
Age, years	1.08 (1.05, 1.11)	<0.001	1.07 (1.05, 1.09)	<0.001
⩾High school education	0.81 (0.58, 1.13)	0.20		
Race				
Caucasian	Referent			
African American	0.80 (0.56, 1.15)	0.23		
Other	0.31 (0.09, 1.11)	0.07		
Smoking				
Never	Referent		Ref.	
Former	1.34 (0.91, 1.97)	0.14	1.46 (1.10, 1.94)	0.009
Current	2.39 (1.66, 3.43)	<0.001	2.17 (1.36, 3.47)	0.001
BMI, kg/m^2	0.97 (0.94, 0.99)	0.007		
BMI <23	0.87 (0.82, 0.93)	<0.001	0.82 (0.71, 0.93)	0.003
BMI >23	0.99 (0.96, 1.02)	0.69	0.99 (0.95, 1.03)	0.53
Comorbidities				
Diabetes	1.38 (1.17, 1.62)	<0.001		
Hypertension	1.07 (0.81, 1.41)	0.65		
Hyperlipidaemia	0.93 (0.64, 1.35)	0.69		
Chronic kidney disease	2.50 (1.64, 3.82)	<0.001	2.56 (1.74, 3.78)	<0.001
Cardiovascular disease	1.52 (1.19, 1.94)	0.001	1.42 (1.09, 1.86)	0.01
Cerebrovascular disease	1.88 (1.23, 2.87)	0.004		
RA-related factors				
Disease duration, years	1.00 (1.00, 1.01)	0.43		
ACPA-positive	1.02 (0.71, 1.49)	0.89		
RF-positive	1.35 (1.02, 1.80)	0.04		
RAPID-3	1.60 (1.44, 1.78)	<0.001	1.51 (1.34, 1.70)	<0.001
DAS-28	1.33 (1.20, 1.46)	<0.001		
CRP, mg/l	1.01 (1.01, 1.02)	<0.001	1.01 (1.00, 1.01)	0.007
Nodules	1.24 (0.82, 1.87)	0.32		
MTX use	0.42 (0.32, 0.54)	<0.001	0.35 (0.23, 0.53)	<0.001
Prednisone use	1.56 (1.13, 2.16)	0.007		
Biologic use	0.45 (0.37, 0.55)	<0.001	0.41 (0.34, 0.51)	<0.001

RAPID: routine assessment of patient index data. Biologics included anti-TNF agents (adalimumab, etanercept and infliximab), abatacept and rituximab.

ApoE4, and the ApoE receptor confer a greater risk of developing atherosclerosis. Ulrich and colleagues [32] recently demonstrated that ApoE4 interferes with the action of ApoE3 to promote the beneficial effects of nitric oxide in endothelial cells *in vitro* and *in vivo*. Potential mechanisms for vascular compromise include protein modification of ApoE by citrullination or anti-Cit-ApoE antibody–antigen immune complexes, leading to abnormal receptor signalling. Our findings suggest that further investigation in this area is merited.

These novel observations add to the growing literature to support the potential role of select ACPA in CVD pathogenesis [11, 33]. Sokolove and colleagues [11] recently demonstrated the presence of citrullinated fibrinogen in atherosclerotic plaque as well as associations of both anti-Cit-fib and anti-Cit-vim with coronary calcium scores in RA patients. These same ACPA were associated with vascular calcifications based on the initial SAM screen in the present study. Antibodies to citrullinated forms of fibrinogen, vimentin and ApoE have also been found to be associated with measures of vascular function in RA [33, 34]. Studies have examined the paradoxical effects of diminished bone mineralization and increased vascular calcifications in the presence of systemic inflammation [35, 36]. Harre and colleagues [37] recently observed that anti-Cit-vim antibodies promoted osteoclastogenesis and bone resorption assisted by TNFα. Further studies are required to determine whether autoantibodies may be directly involved in vascular mineralization.

Our study demonstrated a striking and unexpected inverse relationship between cigarette smoking and the presence of vascular calcifications. Current and former smokers were 70–80% less likely to have radiographic vascular calcifications than never smokers. These findings are inconsistent with recent preventive medicine and population-based studies reporting on risk factors for detectable vascular calcifications [38]. It is possible that the discrepancy of our data with others may be due to statistical truncation where observations of smoking patients are unavailable due to death occurring before study enrolment, so-called left censoring [39]. Other possible explanations include statistical chance, given the low percentage of current smokers in the vascular calcification group, or other yet to be defined biological effects of smoking. Plaque morphology ranges from stable calcifications to unstable soft, fatty plaque, both leading to increased mortality. Smoking may be associated with the latter, which is more prone to rupture and thus carry a higher risk of death [40]. Smoking has also been associated with lower levels of disease activity in patients with ulcerative colitis and Behçet's disease [41, 42]. Whether these effects from smoking could mitigate the formation of radiographically detectable vascular calcifications in the context of RA remains unknown. Lastly, we found prednisone to be positively associated with vascular calcifications, which may be reflective of systemic inflammation related to disease activity. Alternatively, prednisone may be directly involved in the pathogenesis of vessel wall damage [43].

Strengths of this study include the unique availability of radiographic films from a single integrated health care system, the availability of longitudinal clinical data and the statistical power afforded by the large sample size. Further strengths include a diagnosis of RA made by a rheumatologist, as well as the utilization of a single laboratory for ACPA subtypes and cytokine determinations.

As detailed above, cardiovascular events and cause-specific mortality, including CVD-related death, were not available for this study. Additionally, radiographs of the feet were not routinely available, a noteworthy limitation given previous observations of higher rates of radiographic vascular calcifications in the feet compared with the hands in a RA subcohort from this population [44]. Given the lack of standardized algorithms for measuring calcification burden, we did not examine the effect of calcification severity on mortality events. The present effort was limited to U.S. veterans, a population that is unique relative to other RA populations because it comprises predominantly older men with high rates of comorbid illness. Thus, these results may not be generalizable to other populations, particularly women, who account for the largest demographic group affected by RA. However, preliminary data from a predominately female RA cohort showed the same frequency (11%) of vascular calcifications on hand radiographs in patients with prevalent CVD risk factors [45].

In this study, vascular calcifications were evident on hand radiographs in approximately 1 in every 10 RA patients, and their presence was associated with an ~40% increase in all-cause mortality risk. The role that vascular calcifications may play in mortality, such as driving endothelial dysfunction or the consequence of a systemic inflammatory process in RA, has yet to be elucidated. Given their independent associations with vascular calcifications, future studies will be needed to elucidate the potential role of IL-4 and select ACPA (including anti-Cit-ApoE) in atherosclerosis.

Acknowledgements

The VARA registry has received research support from the Health Services Research & Development (HSR&D) Program of the Veterans Health Administration in addition to unrestricted research funds from Abbott Laboratories and Bristol-Myers Squibb. T.R.M. receives research support from the Veterans Health Administration (VA Merit) and the American College of Rheumatology Research and Education Foundation. L.C. is funded by a VA HSR&D Career Development Award. K.D.M. is funded by the Rheumatology Research Foundation Investigator Award. E.B.S. has received research support from the American Heart Association, National Center for Advancing Translational Sciences of the National Institutes of Health, and Bristol-Myers Squibb.

Funding: This work was supported by the Nebraska Arthritis Outcomes Research Centre at the University of Nebraska Medical Centre.

Disclosure statement: K.D.M. receives funding from the Rheumatology Research Foundation Investigator Award. All other authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at *Rheumatology* Online.

References

1 Gabriel SE. Cardiovascular morbidity and mortality in rheumatoid arthritis. Am J Med 2008;121(10 Suppl 1):S9–14.

2 Wolfe F, Mitchell D, Sibley J *et al*. The mortality of rheumatoid arthritis. Arthritis Rheum 1994;37:481–94.

3 Niskanen L, Siitonen O, Suhonen M, Uusitupa MI. Medial artery calcification predicts cardiovascular mortality in patients with NIDDM. Diabetes Care 1994;17:1252–6.

4 London GM, Guerin AP, Marchais SJ *et al*. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. Nephrol Dial Transplant 2003;18:1731–40.

5 Chow BJ, Wells GA, Chen L *et al*. Prognostic value of 64-slice cardiac computed tomography severity of coronary artery disease, coronary atherosclerosis, and left ventricular ejection fraction. J Am Coll Cardiol 2010;55:1017–28.

6 Giles JT, Szklo M, Post W *et al*. Coronary arterial calcification in rheumatoid arthritis: comparison with the Multi-Ethnic Study of Atherosclerosis. Arthritis Res Ther 2009;11:R36.

7 Wang S, Yiu KH, Mok MY *et al*. Prevalence and extent of calcification over aorta, coronary and carotid arteries in patients with rheumatoid arthritis. J Intern Med 2009;266:445–52.

8 Mohammad A, Lohan D, Bergin D *et al*. Vertebral fracture assessment-detected abdominal aortic calcification and cardiovascular disease in rheumatoid arthritis. Semin Arthritis Rheum 2014;43:632–7.

9 del Rincon I, Freeman GL, Haas RW, O'Leary DH, Escalante A. Relative contribution of cardiovascular risk factors and rheumatoid arthritis clinical manifestations to atherosclerosis. Arthritis Rheum 2005;52:3413–23.

10 Gonzalez-Gay MA, Garcia-Unzueta MT, Berja A *et al*. Short-term effect of anti-TNF-alpha therapy on nitric oxide production in patients with severe rheumatoid arthritis. Clin Exp Rheumatol 2009;27:452–8.

11 Sokolove J, Brennan MJ, Sharpe O *et al*. Brief report: citrullination within the atherosclerotic plaque: a potential target for the anti-citrullinated protein antibody response in rheumatoid arthritis. Arthritis Rheum 2013;65:1719–24.

12 Mikuls TR, Fay BT, Michaud K *et al*. Associations of disease activity and treatments with mortality in men with rheumatoid arthritis: results from the VARA Registry. Rheumatology 2011;50:101–9.

13 Mikuls TR, Kazi S, Cipher D *et al*. The association of race and ethnicity with disease expression in male US veterans with rheumatoid arthritis. J Rheumatol 2007;34:1480–4.

14 Arnett F, Edworthy S, Bloch D *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.

15 Wolfe F, Michaud K. Effect of body mass index on mortality and clinical status in rheumatoid arthritis. Arthritis Care Res 2012;64:1471–9.

16 Pincus T, Swearingen CJ, Bergman M, Yazici Y. RAPID3 (Routine Assessment of Patient Index Data 3), a rheumatoid arthritis index without formal joint counts for routine care: proposed severity categories compared to disease activity score and clinical disease activity index categories. J Rheumatol 2008;35:2136–47.

17 Pincus T, Swearingen C, Wolfe F. Toward a multidimensional Health Assessment Questionnaire (MDHAQ): assessment of advanced activities of daily living and psychological status in the patient-friendly health assessment questionnaire format. Arthritis Rheum 1999;42:2220–30.

18 Prevoo ML, van 't Hof MA, Kuper HH *et al*. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44–8.

19 Sokolove J, Johnson DS, Lahey LJ *et al*. Rheumatoid factor as a potentiator of anti-citrullinated protein antibody-mediated inflammation in rheumatoid arthritis. Arthritis Rheumatol 2014;66:813–21.

20 Libby P. Role of inflammation in atherosclerosis associated with rheumatoid arthritis. Am J Med 2008;121(10 Suppl 1):S21–31.

21 Bili A, Tang X, Pranesh S *et al*. Tumor necrosis factor alpha inhibitor use and decreased risk for incident coronary events in rheumatoid arthritis. Arthritis Care Res 2014;66:355–63.

22 Choi HK, Hernan MA, Seeger JD, Robins JM, Wolfe F. Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. Lancet 2002;359:1173–7.

23 Rho YH, Chung CP, Oeser A *et al*. Inflammatory mediators and premature coronary atherosclerosis in rheumatoid arthritis. Arthritis Rheum 2009;61:1580–5.

24 Dixon WG, Watson KD, Lunt M *et al*. Reduction in the incidence of myocardial infarction in patients with rheumatoid arthritis who respond to anti-tumor necrosis factor alpha therapy: results from the British Society for Rheumatology Biologics Register. Arthritis Rheum 2007;56:2905–12.

25 de Boer OJ, van der Wal AC, Verhagen CE, Becker AE. Cytokine secretion profiles of cloned T cells from human aortic atherosclerotic plaques. J Pathol 1999;188:174–9.

26 Engelbertsen D, Andersson L, Ljungcrantz I *et al*. T-helper 2 immunity is associated with reduced risk of myocardial infarction and stroke. Arterioscler Thromb Vasc Biol 2013;33:637–44.

27 Zhou X, Paulsson G, Stemme S, Hansson GK. Hypercholesterolemia is associated with a T helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apo E-knockout mice. J Clin Invest 1998;101:1717–25.

28 Hofbauer LC, Schrader J, Niebergall U *et al*. Interleukin-4 differentially regulates osteoprotegerin expression and induces calcification in vascular smooth muscle cells. Thromb Haemost 2006;95:708–14.

29 Lewis DB, Liggitt HD, Effmann EL *et al*. Osteoporosis induced in mice by overproduction of interleukin 4. Proc Natl Acad Sci U S A 1993;90:11618–22.

30 Bucay N, Sarosi I, Dunstan CR *et al*. *osteoprotegerin*-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 1998;12:1260–8.

31 Curtiss LK, Boisvert WA. Apolipoprotein E and atherosclerosis. Curr Opin Lipidol 2000;11:243–51.

32 Ulrich V, Konaniah ES, Herz J *et al*. Genetic variants of ApoE and ApoER2 differentially modulate endothelial function. Proc Natl Acad Sci U S A 2014;111:13493–8.

33 Bathon J, Giles J, Sokolove J, Robinson W, Geraldino-Pardilla L. Fine-specificity of anti-citrullinated peptide auto-antibodies: associations with cardiac structure and function in rheumatoid arthritis. Arthritis Rheum 2012; 64(Suppl 10):2539 [Abstract].

34 El-Barbary AM, Kassem EM, El-Sergany MA, Essa SA, Eltomey MA. Association of anti-modified citrullinated vimentin with subclinical atherosclerosis in early rheumatoid arthritis compared with anti-cyclic citrullinated peptide. J Rheumatol 2011;38:828–34.

35 Hjortnaes J, Butcher J, Figueiredo JL *et al*. Arterial and aortic valve calcification inversely correlates with osteoporotic bone remodelling: a role for inflammation. Eur Heart J 2010;31:1975–84.

36 den Uyl D, Nurmohamed MT, van Tuyl LH, Raterman HG, Lems WF. (Sub)clinical cardiovascular disease is associated with increased bone loss and fracture risk; a systematic review of the association between cardiovascular disease and osteoporosis. Arthritis Res Ther 2011;13:R5.

37 Harre U, Georgess D, Bang H *et al*. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. J Clin Invest 2012;122: 1791–802.

38 Odink AE, van der Lugt A, Hofman A *et al*. Risk factors for coronary, aortic arch and carotid calcification; The Rotterdam Study. J Hum Hypertens 2010;24:86–92.

39 McEvoy JW, Blaha MJ, Rivera JJ *et al*. Mortality rates in smokers and nonsmokers in the presence or absence of coronary artery calcification. JACC Cardiovasc Imaging 2012;5:1037–45.

40 Missel E, Mintz GS, Carlier SG *et al*. *In vivo* virtual histology intravascular ultrasound correlates of risk factors for sudden coronary death in men: results from the prospective, multi-centre virtual histology intravascular ultrasound registry. Eur Heart J 2008;29:2141–7.

41 Kalayciyan A, Orawa H, Fimmel S *et al*. Nicotine and biochanin A, but not cigarette smoke, induce anti-inflammatory effects on keratinocytes and endothelial cells in patients with Behcet's disease. J Invest Dermatol 2007;127:81–9.

42 Lakhan SE, Kirchgessner A. Anti-inflammatory effects of nicotine in obesity and ulcerative colitis. J Transl Med 2011;9:129.

43 Leitman DC, Benson SC, Johnson LK. Glucocorticoids stimulate collagen and noncollagen protein synthesis in cultured vascular smooth muscle cells. J Cell Biol 1984; 98:541–9.

44 Whiddon AP, Snow MH, Michaud K *et al*. Vascular calcifications on routine hand and foot radiographs of rheumatoid arthritis patients. Arthritis Rheum 2008; 58(Suppl):s284 [Abstract].

45 Mohammad A, English C, Lohan D *et al*. Vascular calcification on hand and feet X-rays, VFA imaging of the spine, and cardiovascular disease in rheumatoid arthritis. Arthritis Rheumatol 2012;64(Suppl 10):1255 [Abstract].