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


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.
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 (κ = 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 (> high school vs < high school), smoking status at enrolment.
knot at 23 kg/m² as a threshold, the latter based on prior
ined as a continuous variable with and without a spline
risk factors present at enrolment, using the
to demographic characteristics and the presence of CVD
Statistical analysis
using banked serum from enrolment.
lyte concentrations (picograms per millilitre) were interpo-
dated using Bio-Plex Manager 5.0 software, and ana-
dies in multiplex immunoassays [19]. Data processing was
demonstrated to reduce the effects of heterophilic antibo-
assay dilution buffer was modified to contain reagents
plex Assay (Bio-Rad, Hercules, CA, USA) run on a
performed using a Bio-Plex Pro Human Cytokines 17-
cubation with anti-human phycoerythrin-labelled anti-
putative RA-associated autoantigens, followed by in-
clusion, with the exception that a proprietary Bio-Rad
platform [11, 19]. Briefly, serum was diluted and mixed
with spectrally distinct fluorescent beads conjugated
putative RA-associated autoantigens, followed by in-
cubation with anti-human phycoerythrin-labelled anti-
body, and analysed on a Luminex 200 instrument.

Autoantibody and cytokine assays
ACPAs were measured using a second generation anti-CCP
antibody ELISA (Diastat, Axis-Shield Diagnostics Ltd,
Dundee, UK; positive if ≥ 5 U/ml), while RF (positive if
≥ 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
putative RA-associated autoantigens, followed by in-
cubation with anti-human phycoerythrin-labelled anti-
body, 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 in-
structions, with the exception that a proprietary Bio-Rad
assay dilution buffer was modified to contain reagents
demonstrated to reduce the effects of heterophilic antibo-
dies in multiplex immunoassays [19]. Data processing was
performed using Bio-Plex Manager 5.0 software, and analy-
concentrations (picograms per millilitre) were interpo-
lated from standard curves. All analyses were performed
using banked serum from enrolment.

Statistical analysis
Patients with and without evidence of radiographically de-
tected vascular calcifications were compared with regard
to demographic characteristics and the presence of CVD
risk factors present at enrolment, using the t-test for con-
tinuous variables and the chi-squared test for categorical
variables. The association of patient characteristics and
RA-related disease features with the presence of radio-
graphic vascular calcifications was subsequently exam-
ined 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 compari-
sons. 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, identi-
ified via initial SAM screening, with the presence of vascu-
lar calcifications. Using this approach, median values of
cytokines/chemokines and ACPAs were modelled as in-
dependent 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 hyperten-
sion, 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 propor-
tional hazards regression with initial age adjustment and
stratified by gender, followed by multivariable adjust-
ments. RA-related measures, including measures of dis-
ease 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 ab-
straction of the VA computerized patient record system,
by next-of-kin notification or by periodic regional VA sur-
villance 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 cal-
cifications and mortality in subjects with diabetes, we esti-
mated two separate models, treating those with and
without diabetes as separate samples. We then per-
formed univariate analysis separately on our two sub-
samples, followed by backwards stepwise selection with
the two different variable lists that came from the univari-
ate analysis. Cause-specific mortality data were not avail-
able for this analysis. All analyses were completed using
Stata version 12 (StataCorp, College Station TX, USA).

Results
Vascular calcifications were observed in hand radio-
graphs 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
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 sub-specificities and cytokines/chemokines were over-represented 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
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 over-expressing 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  

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].

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vascular calcifications present (n = 99)</th>
<th>Vascular calcifications absent (n = 807)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics and health behaviours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean (s.d.), years</td>
<td>72 (9)</td>
<td>64 (11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>96 (97)</td>
<td>725 (90)</td>
<td>0.02</td>
</tr>
<tr>
<td>≥ High school education, n (%)</td>
<td>75 (82)</td>
<td>654 (86)</td>
<td>0.40</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>79 (80)</td>
<td>615 (76)</td>
<td>0.53</td>
</tr>
<tr>
<td>African American</td>
<td>13 (13)</td>
<td>142 (18)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>7 (7)</td>
<td>50 (6)</td>
<td></td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>38 (38)</td>
<td>146 (18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Former</td>
<td>53 (54)</td>
<td>427 (53)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>8 (8)</td>
<td>234 (29)</td>
<td></td>
</tr>
<tr>
<td>BMI, mean (s.d.), kg/m²</td>
<td>27 (5)</td>
<td>28 (5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Comorbidity, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>38 (38)</td>
<td>139 (17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>67 (68)</td>
<td>464 (58)</td>
<td>0.05</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>45 (45)</td>
<td>343 (43)</td>
<td>0.58</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>12 (12)</td>
<td>43 (5)</td>
<td>0.008</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>43 (43)</td>
<td>164 (20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>7 (7)</td>
<td>57 (7)</td>
<td>0.99</td>
</tr>
<tr>
<td>RA-related factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration, mean (s.d.), years</td>
<td>16 (13)</td>
<td>13 (11)</td>
<td>0.004</td>
</tr>
<tr>
<td>ACPA-positive, n (%)</td>
<td>74 (77)</td>
<td>620 (78)</td>
<td>0.79</td>
</tr>
<tr>
<td>RF-positive, n (%)</td>
<td>73 (75)</td>
<td>635 (80)</td>
<td>0.26</td>
</tr>
<tr>
<td>RAPID-3 score, mean (s.d.)</td>
<td>2.9 (1.3)</td>
<td>2.5 (1.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>DAS-28 score, mean (s.d.)</td>
<td>3.9 (1.5)</td>
<td>3.8 (1.5)</td>
<td>0.29</td>
</tr>
<tr>
<td>CRP, mean (s.d.), mg/l</td>
<td>14 (24)</td>
<td>11 (18)</td>
<td>0.21</td>
</tr>
<tr>
<td>Nodules, n (%)</td>
<td>44 (44)</td>
<td>337 (42)</td>
<td>0.61</td>
</tr>
<tr>
<td>MTX use, n (%)</td>
<td>51 (53)</td>
<td>427 (56)</td>
<td>0.52</td>
</tr>
<tr>
<td>Prednisone use, n (%)</td>
<td>53 (55)</td>
<td>313 (41)</td>
<td>0.01</td>
</tr>
<tr>
<td>Biologic use, n (%)</td>
<td>33 (33)</td>
<td>262 (32)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>2.85 (1.43, 5.66)</td>
<td>0.003</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>2.48 (1.01, 6.09)</td>
<td>0.047</td>
</tr>
<tr>
<td>Prednisone use</td>
<td>1.90 (1.25, 2.91)</td>
<td>0.003</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>0.36 (0.27, 0.48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current</td>
<td>0.06 (0.01, 0.23)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
### Table 3: Multivariable quantile regression of ACPA subtypes and cytokines and the associations with radiographic vascular calcifications

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significance analysis of microarray</th>
<th>Multivariable quantile regression, P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fold change VC vs VC</td>
<td>Q-value, %</td>
</tr>
<tr>
<td>ACPA subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-citrullinated ApoE</td>
<td>1.28</td>
<td>0</td>
</tr>
<tr>
<td>Anti-citrullinated vimentin</td>
<td>1.35</td>
<td>0</td>
</tr>
<tr>
<td>Anti-citrullinated fibrinogen</td>
<td>1.30</td>
<td>0</td>
</tr>
<tr>
<td>Cytokine/chemokine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>1.37</td>
<td>0</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.26</td>
<td>0</td>
</tr>
</tbody>
</table>

ACPA subtypes and cytokines generated by significance analysis of microarrays analysis followed by multivariable quantile regression. 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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age-adjusted and gender-stratified univariate HR (95% Cl)</th>
<th>P-value</th>
<th>Multivariable HR (95% Cl)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular calcification</td>
<td>1.36 (1.06, 1.74)</td>
<td>0.02</td>
<td>1.41 (1.12, 1.78)</td>
<td>0.004</td>
</tr>
<tr>
<td>Demographics and health behaviours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>1.08 (1.05, 1.11)</td>
<td>&lt;0.001</td>
<td>1.07 (1.05, 1.09)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥ High school education</td>
<td>0.81 (0.58, 1.13)</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>Referent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>0.80 (0.56, 1.15)</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.31 (0.09, 1.11)</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Referent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>1.34 (0.91, 1.97)</td>
<td>0.14</td>
<td>1.46 (1.10, 1.94)</td>
<td>0.009</td>
</tr>
<tr>
<td>Current</td>
<td>2.39 (1.66, 3.43)</td>
<td>&lt;0.001</td>
<td>2.17 (1.36, 3.47)</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI ≥ 23</td>
<td>0.87 (0.82, 0.93)</td>
<td>&lt;0.001</td>
<td>0.82 (0.71, 0.93)</td>
<td>0.003</td>
</tr>
<tr>
<td>BMI &gt; 23</td>
<td>0.99 (0.96, 1.02)</td>
<td>0.69</td>
<td>0.99 (0.95, 1.03)</td>
<td>0.53</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.38 (1.17, 1.62)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.07 (0.81, 1.41)</td>
<td>0.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>0.93 (0.64, 1.35)</td>
<td>0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>2.50 (1.64, 3.82)</td>
<td>&lt;0.001</td>
<td>2.56 (1.74, 3.78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>1.52 (1.19, 1.94)</td>
<td>0.001</td>
<td>1.42 (1.09, 1.86)</td>
<td>0.01</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>1.88 (1.23, 2.87)</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RA-related factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>1.00 (1.00, 1.01)</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACPA-positive</td>
<td>1.02 (0.71, 1.49)</td>
<td>0.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF-positive</td>
<td>1.35 (1.02, 1.80)</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAPID-3</td>
<td>1.60 (1.44, 1.78)</td>
<td>&lt;0.001</td>
<td>1.51 (1.34, 1.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DAS-28</td>
<td>1.33 (1.20, 1.46)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>1.01 (1.01, 1.02)</td>
<td>&lt;0.001</td>
<td>1.01 (1.00, 1.01)</td>
<td>0.007</td>
</tr>
<tr>
<td>Nodules</td>
<td>1.24 (0.82, 1.87)</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTX use</td>
<td>0.42 (0.32, 0.54)</td>
<td>&lt;0.001</td>
<td>0.35 (0.23, 0.53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prednisone use</td>
<td>1.56 (1.13, 2.16)</td>
<td>0.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologic use</td>
<td>0.45 (0.37, 0.55)</td>
<td>&lt;0.001</td>
<td>0.41 (0.34, 0.51)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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]. Haar 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.

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Supplementary data
Supplementary data are available at Rheumatology Online.

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