Concise report

Association of anti-citrullinated protein or peptide antibodies with left ventricular structure and function in rheumatoid arthritis

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Abstract

Objective. High levels of ACPAs in RA are associated with more severe arthritis and worse prognosis. However, the role of ACPAs in mediating the increased risk of heart failure in RA remains undefined. We examined whether specific ACPAs were associated with subclinical left ventricular (LV) phenotypes that presage heart failure.

Methods. Sera from RA patients without clinical cardiovascular disease were assayed for specific ACPAs using a custom Bio-Plex bead assay, and their cross-sectional associations with cardiac magnetic resonance-derived LV measures were evaluated. High ACPA level was defined as ≥ 75th percentile. Findings were assessed in a second independent RA cohort with an expanded panel of ACPAs and LV measures assessed by 3D-echocardiography.

Results. In cohort 1 (n = 76), higher levels of anti-citrullinated fibrinogen⁴¹-⁶⁰ and anti-citrullinated vimentin antibodies were associated with a 10 and 6% higher adjusted mean LV mass index (LVMI), respectively, compared with lower antibody levels (P < 0.05). In contrast, higher levels of anti-citrullinated biglycan²⁴⁷-²⁶⁶ were associated with a 13% lower adjusted mean LVMI compared with lower levels (P < 0.001). In cohort 2 (n = 74), the association between ACPAs targeting citrullinated fibrinogen and citrullinated vimentin peptides or protein and LVMI was confirmed: higher anti-citrullinated fibrinogen⁵⁵⁶-⁵⁷⁵ and anti-citrullinated vimentin⁵⁸⁸-⁷⁷ antibody levels were associated with a higher adjusted mean LVMI (19 and 15%, respectively; P < 0.05), but no association with biglycan was found.

Conclusion. Higher levels of antibodies targeting citrullinated fibrinogen and vimentin peptides or protein were associated with a higher mean LVMI in both RA cohorts, potentially implicating autoimmune targeting of citrullinated proteins in myocardial remodelling in RA.

Key words: anti-citrullinated peptide antibody (ACP A), RA, ventricular mass, CVD

Rheumatology key messages

- Higher levels of ACPAs targeting citrullinated fibrinogen and citrullinated vimentin peptides or protein were associated with higher left ventricular mass index.
- Seroreactivity towards citrullinated proteins may play a role in myocardial remodelling in RA.
Introduction

Cardiovascular disease (CVD) represents the leading cause of mortality in RA [1]. RA patients have double the risk for developing heart failure compared with controls [1] even after adjusting for traditional CV risk factors and ischaemic heart disease, suggesting that RA is an independent risk factor for heart failure. Although the excess in myocardial dysfunction in RA remains unexplained, autopsy studies show myocarditis in some patients [2]. Differences in symptoms, myocardial phenotypes, and higher heart failure-related mortality rates in RA vs non-RA patients [3] suggest different mechanisms for myocardial dysfunction in RA vs controls.

ACPAs are a key feature of, and relatively specific for, RA, appearing in the pre-clinical phase of the disease [4]. Whether the citrullinated autoantigens identified from synovium and recognized by RA sera [5–7] are found in the myocardium and contribute to myocardial dysfunction in RA remains unexplored. In RA necropsied myocardia, we reported higher citrullination levels relative to autoimmune and non-autoimmune disease controls, and confirmed myocardial expression of the citrullinating enzyme, peptidyl arginine deiminase [8]. However, the identity of the citrullinated myocardial proteins was not established. Some of the citrullinated autoantigens discovered in RA synovium (vimentin, biglycan, fibronectin) [9] are expressed in their native state in myocardial tissue, raising the possibility that ACPAs against their citrullinated myocardial counterparts may be generated in RA and induce myocardial remodelling.

Using an array of RA-associated autoantigens, we investigated the association of ACPAs with parameters of left ventricular (LV) structure and function in two RA cohorts without clinical CVD. We hypothesized that patients with high ACPA levels would have different myocardial phenotypes from patients with lower antibody levels.

Methods

Patients

Cohort 1 included 76 patients in the Evaluation of Subclinical CArdiovascular disease and Predictors of Events in RA (ESCAPE-RA) study, randomly selected to undergo cardiac MRI [10] in addition to cardiovascular phenotyping. Participants were 45–84 years old, met 1987 ACR RA criteria [11], had RA for ≥6 months, and had no clinical CVD (defined as coronary artery disease, myocardial infarction, heart failure, stroke). Cohort 2 included the first 74 enrollees in RHematic arthritis Myocardium (RHYTHM), an ongoing study of subclinical myocardial phenotypes in RA patients without CVD in which all participants underwent 3D-echocardiography. RHYTHM inclusion/exclusion criteria were identical to ESCAPE-RA except for an age ≥18 years. Studies were approved by the Johns Hopkins Medical Institutions (ESCAPE-RA) and Columbia University (RHYTHM) Institutional Review Boards, which included approval for this study. Informed consent was obtained for both the ESCAPE-RA and RHYTHM studies.

Outcome measures

Cardiac MRI

Cardiac MRI (CMR) in ESCAPE-RA was performed using a 1.5-T magnet CV/i (GE Healthcare, Piscataway, NJ, USA). Ejection fraction, stroke volume and body surface area (BSA)-indexed end-diastolic and end-systolic volumes, and left ventricular mass (LVM) were analysed as previously described [10].

3D echocardiography

RHYTHM participants underwent transthoracic 3D-echocardiography using the Philips IE 33 system. 3D-LV volumes and mass were measured using the Philips QLAB Advanced Quantification software 8.1; LV end-diastolic and end-systolic volumes were indexed by BSA. LVM was calculated as the difference between the epicardial and endocardial volumes multiplied by the specific myocardial tissue mass and indexed by BSA. The LV mass-to-end-diastolic volume (M/V) ratio was used as a parameter of LV geometry.

Laboratory assays

Phlebotomy was performed at the same visit as the CMR or 3D-echoangiography. Serum and plasma were centrifuged and stored at −70°C.

Anti-CCP assays

Anti-CCP-antibodies were assessed by ELISA (CCP2 kit, for ESCAPE-RA, and CCP3 kit for RHYTHM; Inova Diagnostics, Woburn, MA, USA), with positivity defined as ≥60 U.

ACPA assays

A multiplex platform, utilizing a custom Bio-Plex bead-based autoantibody assay, was used for the analysis of autoantibodies targeting specific RA-associated citrullinated and native (non-citrullinated) protein or peptides, as previously described [5]. A high ACPA level was defined as ≥75th percentile. For ESCAPE-RA, the array consisted of 17 citrullinated and three native proteins or peptides [6]. For RHYTHM, 30 citrullinated and five native proteins or peptides were measured [7]. Intra-assay and interassay coefficients of variance ranged from 0.4 to 5.5% and 0.9 to 10%, respectively.

Additional assays

High-sensitivity CRP, IL-6 and lipid levels were measured as previously described [10, 12]. RF was assessed by ELISA, with positivity defined as ≥40 U.

Clinical covariates

Demographics, smoking and CVD family history were collected from identical questionnaires for both cohorts. Hypertension, diabetes, BMI, BSA, medication use and RA disease duration and activity were ascertained as previously described [10, 12].
Statistical methods

Summary statistics were examined; comparisons were made using Student’s t test and Wilcoxon’s rank-sum test for normally and non-normally distributed continuous variables, respectively. Counts and percentages were calculated for categorical variables, compared using the chi-square or Fisher’s exact test. Multivariable linear regression was used to model the association of LV structure and function measures with the panel of seroreactivities towards citrullinated and non-citrullinated autoantigens. Tolerance was calculated to avoid modelling collinear variables. Confounders were defined as variables associated with both the outcome (cardiac structure and function measures) and predictors (ACPAs levels). Statistical calculations were performed using SAS 9.4 (SAS Institute, Cary, NC, USA). A two-tailed \( \alpha \) of 0.05 was defined as the level of statistical significance.

Results

Patient characteristics

Patient characteristics are summarized in Table 1. In ESCAPE-RA, the mean age was 59 years with 51% females and 85% self-identified as white. RHYTHM participants had a mean age of 54 years, 85% females and 36% whites. In both studies, the median disease duration was 7 years, two-thirds were anti-CCP antibody positive and mean RA disease activity was moderate. Hypertension and diabetes were more frequent in RHYTHM patients.

With anti-CCP-antibody level dichotomized at the 75th percentile, in ESCAPE-RA a lower BMI and higher percentage of smokers were noted in the high anti-CCP group. In RHYTHM, more patients with RF >40 U and a trend towards higher prednisone use were observed in the high anti-CCP group. ESCAPE-RA patients in the high anti-CCP group had a lower stroke volume and a lower mean LVMI, as previously published, although not reaching statistical significance in this analysis (due to analysing anti-CCP as a categorical rather than continuous variable). In RHYTHM, LVMI was not different between anti-CCP group had a lower stroke volume and a lower mean LVMI, as previously published, although not reaching statistical significance in this analysis (due to analysing anti-CCP as a categorical rather than continuous variable). In RHYTHM, LVMI was not different between anti-CCP groups, although a trend towards a higher LVMI in the high anti-CCP-antibody group was seen. Given this discrepancy, specific ACPAs were examined.

Association of ACPAs with LV parameters

The citrullinated and non-citrullinated proteins or peptides that comprised the autoantigen microarrays are listed in Table 2.

ESCAPE-RA

Higher anti-citrullinated (cit) fibrinogen556–575, anti-cit-vimentin58–77 and anti-cit-fibronectin1029–1042 antibody levels were associated with a higher mean LVMI compared with lower antibody levels. In contrast, high anti-cit-biglycan247–266 antibody levels were associated with a lower mean LVMI compared with lower antibody levels. After adjusting for confounders, these associations remained significant.

RHYTHM

Higher anti-cit-fibrinogen556–575, anti-cit-vimentin58–77 and anti-cit-fibronectin1029–1042 antibody levels were associated with a higher mean LVMI compared with lower antibody levels in unadjusted and adjusted analyses. Higher anti-citrullinated biglycan247–266 levels were not associated with LVMI in this cohort.

In both cohorts, LV M/V ratios were normal (suggesting an eccentric LV geometry) with no differences noted per anti-CCP or ACPA strata (\( P > 0.05 \)). Other measured cardiac parameters were not associated with specific ACPA reactivities in either cohort (data not shown). No association was seen between LVMI and levels of seroreactivity to the non-citrullinated protein controls in either cohort.

Discussion

This is the first investigation of the association of specific ACPAs with LV structure, function and geometric parameters in RA patients. We found in two independent RA cohorts, despite different demographics and imaging methodologies, that high levels of ACPAs targeting cit-vimentin and cit-fibrinogen protein or peptides were associated with higher mean LVMI. While only evaluated in the RHYTHM participants, a similar association was found for ACPAs targeting cit-fibronectin peptides. The specificity of these responses was confirmed by lack of association of LVMI with antibody levels against non-citrullinated homologue proteins.

Increased LVM is the most common form of adverse LV remodelling, considered a subclinical phenotype in the pathway to heart failure development [13]. In the Framingham Study, for every 50 g increase in LVM, CVD increased by 1.49- and 1.57-fold for men and women, respectively, with higher relative risks for cardiovascular death of 1.73 and 2.12, respectively [14]. In our studies, although the higher LVMI noted in the RA patients with high-ACPA levels fell within the normal range, a progressive increase in LVM is a heart failure precursor in the general population, and strong sudden cardiac death predictor, even in subsets with normal ejection fraction [13, 14]. A recent meta-analysis of cross-sectional studies in RA patients without clinical heart failure revealed no consensus on LVMI differences between RA and controls, as higher LVMI, lower LVMIs and no difference in LVMI have all been reported [10, 15]. This could be due to variability in time of assessment along the continuum of the RA disease process. Unfortunately, longitudinal measurements of LVMI in RA patients transitioning from pre-clinical to clinical heart failure are lacking.

Correlation of specific ACPAs with myocardial phenotypes in RA may deepen the understanding of disease pathogenesis through identification of potentially relevant autoantigens in LV impairment. Commercially available anti-CCP assays result in mass-averaging of the ACPA response and do not provide information about the antibody response sub-specificities. Indeed, our studies using two different commercial anti-CCP assays showed no consistent relationship between anti-CCP titres and...
## Table 1  Patient characteristics according to cohort and anti-CCP status

<table>
<thead>
<tr>
<th></th>
<th>ESCAPE Total (n = 76)</th>
<th>ESCAPE Lower CCP Ab (n = 57)</th>
<th>ESCAPE Higher CCP Ab (n = 19)</th>
<th>P-value</th>
<th>RHYTHM Total (n = 74)</th>
<th>RHYTHM Lower CCP Ab (n = 55)</th>
<th>RHYTHM Higher CCP Ab (n = 19)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, mean (SD), years</strong></td>
<td>59 (9)</td>
<td>59 (9)</td>
<td>61 (9)</td>
<td>0.28</td>
<td>54 (13)</td>
<td>53 (13)</td>
<td>56 (14)</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>Female, n (%)</strong></td>
<td>39 (51)</td>
<td>29 (51)</td>
<td>10 (53)</td>
<td>0.89</td>
<td>63 (89)</td>
<td>49 (89)</td>
<td>14 (74)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Non-Hispanic white, n (%)</strong></td>
<td>65 (86)</td>
<td>49 (86)</td>
<td>16 (84)</td>
<td>0.85</td>
<td>27 (36)</td>
<td>22 (40)</td>
<td>5 (26)</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>RA duration, median (range), years</strong></td>
<td>7 (3-17)</td>
<td>7 (4-16)</td>
<td>11 (5-19)</td>
<td>0.38</td>
<td>7 (3-17)</td>
<td>8 (3-14)</td>
<td>7 (1-15)</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>DAS28-CRP, median (IQR), units</strong></td>
<td>3.2 (2.7-4.7)</td>
<td>3.2 (2.7-4.1)</td>
<td>3.7 (2.8-4.3)</td>
<td>0.34</td>
<td>3.9 (2.9-4.5)</td>
<td>3.9 (3.0-4.4)</td>
<td>4.5 (2.6-4.8)</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>HAQ, median (IQR), units</strong></td>
<td>0.7 (0.1-1.3)</td>
<td>0.87 (0.1-1.3)</td>
<td>0.25 (0-1.25)</td>
<td>0.16</td>
<td>1 (0.5-1.75)</td>
<td>0.9 (0.4-1.5)</td>
<td>1.2 (1-1.8)</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>IL-6, median (IQR), ng/ml</strong></td>
<td>2.0 (1.1-3.0)</td>
<td>1.9 (0.9-3.8)</td>
<td>3.5 (1.9-6.6)</td>
<td>0.22</td>
<td>2.7 (0-6.6)</td>
<td>2.2 (0.6-6.6)</td>
<td>2.8 (1.2-6.3)</td>
<td>0.72</td>
</tr>
<tr>
<td><strong>Current non-biologic use, n (%)</strong></td>
<td>67 (88)</td>
<td>51 (89)</td>
<td>16 (84)</td>
<td>0.68</td>
<td>51 (73)</td>
<td>38 (72)</td>
<td>13 (76)</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Current biologic use, n (%)</strong></td>
<td>37 (49)</td>
<td>26 (46)</td>
<td>11 (58)</td>
<td>0.35</td>
<td>23 (33)</td>
<td>17 (32)</td>
<td>6 (35)</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Current prednisone use, n (%)</strong></td>
<td>30 (39)</td>
<td>23 (40)</td>
<td>7 (37)</td>
<td>0.79</td>
<td>24 (34)</td>
<td>15 (28)</td>
<td>9 (53)</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Current prednisone dose, median (IQR), mg/day</strong></td>
<td>2.4 (0-5)</td>
<td>0 (0-5)</td>
<td>0 (0-5)</td>
<td>0.96</td>
<td>5 (4-10)</td>
<td>5 (2-10)</td>
<td>7.5 (5-10)</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Diabetes, n (%)</strong></td>
<td>5 (7)</td>
<td>5 (9)</td>
<td>0 (0)</td>
<td>0.32</td>
<td>8 (11)</td>
<td>6 (11)</td>
<td>2 (12)</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Hypertension, n (%)</strong></td>
<td>27 (35)</td>
<td>21 (37)</td>
<td>6 (32)</td>
<td>0.68</td>
<td>30 (40)</td>
<td>22 (40)</td>
<td>8 (42)</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>SBP, mean (SD), mmHg</strong></td>
<td>129 (17)</td>
<td>128 (16)</td>
<td>133 (20)</td>
<td>0.22</td>
<td>117 (19)</td>
<td>118 (20)</td>
<td>117 (15)</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>DBP, mean (SD), mmHg</strong></td>
<td>77 (9)</td>
<td>76 (9)</td>
<td>79 (9)</td>
<td>0.29</td>
<td>70 (10)</td>
<td>70 (11)</td>
<td>71 (8)</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Anthypertensive agents, n (%)</strong></td>
<td>27 (35)</td>
<td>21 (37)</td>
<td>6 (32)</td>
<td>0.68</td>
<td>27 (36)</td>
<td>19 (34)</td>
<td>8 (42)</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>Total cholesterol, mean (SD), mmol/l</strong></td>
<td>5.0 (1.1)</td>
<td>5.1 (1.1)</td>
<td>4.9 (1.1)</td>
<td>0.52</td>
<td>4.9 (1.5)</td>
<td>4.9 (1.1)</td>
<td>5.0 (0.7)</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>LDL-C, mean (SD), mmol/l</strong></td>
<td>3.0 (0.8)</td>
<td>3.0 (0.9)</td>
<td>3.0 (0.7)</td>
<td>0.97</td>
<td>2.8 (0.7)</td>
<td>2.7 (0.9)</td>
<td>2.9 (0.7)</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>Triglycerides, mean (SD), mmol/l</strong></td>
<td>1.3 (0.7)</td>
<td>1.3 (0.7)</td>
<td>1.1 (0.6)</td>
<td>0.15</td>
<td>1.3 (0.7)</td>
<td>1.3 (0.7)</td>
<td>1.3 (0.8)</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Lipid lowering agents, n (%)</strong></td>
<td>12 (16)</td>
<td>7 (12)</td>
<td>5 (26)</td>
<td>0.15</td>
<td>12 (16)</td>
<td>9 (16)</td>
<td>3 (16)</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Current smoking, n (%)</strong></td>
<td>12 (16)</td>
<td>6 (10)</td>
<td>6 (32)</td>
<td>0.03</td>
<td>28 (36)</td>
<td>22 (41)</td>
<td>8 (47)</td>
<td>0.69</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>27 (9)</td>
<td>28 (5)</td>
<td>25 (4)</td>
<td>0.03</td>
<td>29 (7)</td>
<td>29 (6)</td>
<td>29 (7)</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>CAC score, median (IQR), units</strong></td>
<td>6 (0-215)</td>
<td>5.7 (0-227)</td>
<td>8.6 (0-213)</td>
<td>0.67</td>
<td>0 (0-45)</td>
<td>0 (0-67)</td>
<td>0 (0-45)</td>
<td>0.84</td>
</tr>
<tr>
<td><strong>LV mass, mean (SD), g</strong></td>
<td>120 (26)</td>
<td>123 (27)</td>
<td>113 (22)</td>
<td>0.15</td>
<td>103 (34)</td>
<td>98 (34)</td>
<td>117 (34)</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>LVMI, mean (SD), g/BSA</strong></td>
<td>63 (10)</td>
<td>64 (10)</td>
<td>61 (9)</td>
<td>0.25</td>
<td>60 (16)</td>
<td>59 (17)</td>
<td>66 (14)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>End-diastolic volume index, mean (SD), ml/BSA</strong></td>
<td>64 (12)</td>
<td>66 (12)</td>
<td>60 (8)</td>
<td>0.03</td>
<td>52 (17)</td>
<td>50 (17)</td>
<td>57 (18)</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>End-systolic volume index, mean (SD), ml/BSA</strong></td>
<td>21 (6)</td>
<td>22 (6)</td>
<td>21 (5)</td>
<td>0.73</td>
<td>22 (8)</td>
<td>21 (6)</td>
<td>25 (14)</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>LV M/V ratio, median (IQR), g/ml</strong></td>
<td>1 (0.9-1.1)</td>
<td>1 (1-1.1)</td>
<td>1 (1-1.1)</td>
<td>0.51</td>
<td>1.1 (0.9-1.4)</td>
<td>1.3 (0.9-1.7)</td>
<td>1.2 (0.5-1.8)</td>
<td>0.73</td>
</tr>
<tr>
<td><strong>Stroke volume, mean (SD), ml</strong></td>
<td>80 (19)</td>
<td>82 (20)</td>
<td>72 (15)</td>
<td>0.03</td>
<td>57 (18)</td>
<td>55 (16)</td>
<td>61 (24)</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>Ejection fraction, mean (SD), %</strong></td>
<td>67 (8)</td>
<td>67 (6)</td>
<td>65 (7)</td>
<td>0.23</td>
<td>60 (15)</td>
<td>62 (14)</td>
<td>55 (19)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

BSA: body surface area; CAC: coronary artery calcium; DBP: diastolic blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; LV: left ventricle; LVMI: left ventricular mass index; M/V: mass-to-volume; SBP: systolic blood pressure.
### Table 2: Left ventricular mass index (g/m²) per autoreactivity level in the ESCAPE-RA and RHYTHM cohorts

<table>
<thead>
<tr>
<th>Autoantigen</th>
<th>ESCAPE-RA (n = 76)</th>
<th>RHYTHM (n = 74)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariable model</td>
<td>Multivariable model</td>
</tr>
<tr>
<td></td>
<td>Lower level</td>
<td>Higher level</td>
</tr>
<tr>
<td>Fibrinogen cit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen A 41–60 cit</td>
<td>63 (60, 65)</td>
<td>64 (69, 68)</td>
</tr>
<tr>
<td>Fibrinogen A 556–575 cit</td>
<td>62 (60, 65)</td>
<td>65 (61, 70)</td>
</tr>
<tr>
<td>Fibrinogen A 211–230 cit</td>
<td>63 (60, 66)</td>
<td>63 (59, 68)</td>
</tr>
<tr>
<td>Fibrinogen A 616–635 cit</td>
<td>63 (60, 66)</td>
<td>63 (59, 68)</td>
</tr>
<tr>
<td>Fibrinogen A 582–599 cit</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fibrinogen A 27–43 cit</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fibrinogen B 36–52 cit</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fibrinogen B 54–72 cit</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fibrinogen B 246–267 cit</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Vimentin cit</td>
<td>58 (55, 61)</td>
<td>68 (64, 73)**</td>
</tr>
<tr>
<td>Vimentin 58–77 cit</td>
<td>63 (61, 66)</td>
<td>62 (57, 66)</td>
</tr>
<tr>
<td>Vimentin 1–16 cit</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Apolipoprotein A1 cit</td>
<td>64 (61, 66)</td>
<td>61 (67, 66)</td>
</tr>
<tr>
<td>Apolipoprotein A1 231–248 cit</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Apolipoprotein E cit</td>
<td>63 (61, 66)</td>
<td>62 (67, 67)</td>
</tr>
<tr>
<td>Apolipoprotein E 277–296 cit</td>
<td>63 (60, 65)</td>
<td>64 (69, 68)</td>
</tr>
<tr>
<td>Fibrinogen cit</td>
<td>63 (60, 65)</td>
<td>63 (59, 68)</td>
</tr>
<tr>
<td>Histone 2A cit</td>
<td>67 (62, 70)</td>
<td>68 (64, 70)**</td>
</tr>
<tr>
<td>Histone 2A 1–20 cit</td>
<td>63 (61, 66)</td>
<td>62 (67, 66)</td>
</tr>
<tr>
<td>Histone 2A 1–20 cit sm</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Histone 2B cit</td>
<td>63 (61, 66)</td>
<td>62 (68, 70)</td>
</tr>
<tr>
<td>Histone 2B 2–81 cit</td>
<td>63 (61, 66)</td>
<td>62 (68, 67)</td>
</tr>
<tr>
<td>Clusterin 231–250 cit</td>
<td>63 (60, 65)</td>
<td>64 (69, 68)</td>
</tr>
<tr>
<td>Clusterin 221–240 cit</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Enolase citd</td>
<td>63 (61, 66)</td>
<td>63 (58, 67)</td>
</tr>
<tr>
<td>Enolase A 5–21 cit</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fibronectin 1029–1042 cit</td>
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</table>
LVMI across the two cohorts, in contrast to the consistency observed in the association of high levels of ACPAs targeting cit-fibrinogen and cit-vimentin peptides or proteins and the LVMI. Importantly, all autoantigens identified here are known myocardial constituents and/or are associated with a risk for sudden cardiac death [16/18]. It is therefore possible that citrullination of these proteins might occur in RA hearts and serve as targets for RA-associated ACPAs leading to tissue damage.

Whether citrullination plays a role in myocardial disease in general, or in RA myocardial disease in particular, is a topic of interest. Citrullination is a ubiquitous finding in inflamed tissues [8]. Indeed, although higher in RA hearts, we previously reported citrullination in necropsied hearts of patients with other autoimmune diseases and non-autoimmune myocarditis as well [8]. Recently, in myocardial tissues from non-RA patients with heart failure, we identified a myocardial citrullinated proteome that included citrullinated vimentin and key myofilament structural proteins including citrullinated myosin and tropomyosin [19]. Citrullination of vimentin, a mesenchymal cell intermediate filament component, results in collapse of the filament network, while citrullination of myosin and tropomyosin alters their assembly and contractility patterns [19]. However, the consequences of ACPAs against putative citrullinated myocardial proteins are not known.

Given the specificity of ACPA generation as a feature of RA, it is tempting to speculate that these autoantibodies contribute to the excess risk of heart failure in RA vs non-RA controls. Although a concentric geometry (increased LV M/V ratio) has been previously described in RA-associated increased LVMI, in our study the LVMI increase in high-A CPA groups was associated with eccentric geometry (normal LV M/V ratio), which is commonly seen in primary myocardial injury but remains unknown. The association of ACPAs targeting the same protein or peptides within the protein with the same myocardial phenotype across two RA cohorts despite ethnic differences and two different but well validated and sensitive methods for LV structure and function measurement adds to the robustness of our findings.

Limitations of our study include its cross-sectional nature and testing of multiple comparisons that precludes establishment or testing of multiple comparisons. Also, LVMI was measured by different techniques in the two cohorts; however, a high correlation between these modalities has been established [20]. Finally, the RA microarrays utilized here include a set of synovium-identified autoantigens; thus, potential additional autoantigens of greater myocardial relevance were possibly missed. However, current studies targeting citrullinated antigen and/or citrullinated peptides of potential myocardial relevance are being implemented in the ESCAPE-RA cohort.

**Table 2** Continued

<table>
<thead>
<tr>
<th></th>
<th>ESCAPE-RA (n = 76)</th>
<th>RHYTHM (n = 74)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariable model</td>
<td>Multivariable model&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Lower level</td>
<td>Higher level</td>
</tr>
<tr>
<td></td>
<td>Lower level</td>
<td>Higher level</td>
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<tr>
<td>Non-citrullinated controls</td>
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<td>Fibrinogen</td>
<td>62 (60, 65)</td>
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<td>Apo A1</td>
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<td>66 (61, 70)</td>
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<td>Apo E&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>65 (60, 69)</td>
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<tr>
<td>Vimentin&lt;sup&gt;2&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Histone 2A&lt;sub&gt;0&lt;/sub&gt;</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Histone 2B&lt;sub&gt;0&lt;/sub&gt;</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Mean LVMI (g/m<sup>2</sup>) and 95% CI of low vs high autoreactivities (dichotomized at the 75th percentile). <sup>a</sup>Adjusted for gender, smoking, hypertension and LDL-cholesterol. <sup>b</sup>Adjusted for diastolic blood pressure. <sup>2</sup>Only tested in the RHYTHM cohort. <sup>3</sup>Only tested in the ESCAPE-RA cohort. cit, citrullinated; sm= small peptide. *P < 0.05, **P < 0.01.
are in progress to identify citrullinated myocardial proteins recognized by RA sera.

In conclusion, higher levels of ACPAs targeting citrullinated vimentin and fibrinogen protein or peptides were associated with a higher LVMI compared with lower antibody levels in two RA cohorts without clinical CVD. This association suggests a pathophysiological link between autoimmunity and myocardial remodelling.

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References