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Associations of Galectin-3 Levels With Measures of Vascular Disease in Patients With Rheumatoid Arthritis

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Associations of galectin-3 levels with measures of vascular disease in patients with rheumatoid arthritis

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ABSTRACT

Objectives: Galectin-3 is a beta-galactoside-binding lectin and is a marker of cardiovascular disease (CVD) in the general population. It may also play a role in joint inflammation. We asked whether serum galectin-3 is a useful marker of subclinical vascular disease in patients with rheumatoid arthritis (RA).

Methods: RA patients without clinical CVD underwent assessment of coronary artery calcium (CAC) score, aortic inflammation (using 18Fluorodeoxyglucose positron emission-computed tomography [FDG PET/CT]), and myocardial flow reserve (MFR). Aorta FDG uptake was measured as standardized uptake values (SUV). Generalized linear models were constructed to explore the associations of galectin-3 levels with CAC score, aortic SUV, and MFR.

Results: A total of 124 RA patients (mean age 57; 82 % women, 45 % Hispanic; median RA duration 6.8 years; 75 % seropositive; median CDAI 16; 33 % on prednisone; 89 % on DMARDs; median CAC score 0; median aorta SUV 2.59; mean MFR 2.86; median galectin-3 level 8.54 ng/mL) were analyzed. In univariable analysis, higher galectin-3 levels were associated with higher aortic SUV ($p = 0.007$) but CAC score and MFR were not. In multivariable analysis, higher galectin-3 level remained significantly associated with higher aortic SUV (ß Coefficient=0.1786, p value=0.002).

Conclusion: In our cohort of RA patients without clinical CVD, higher serum galectin-3 levels were independently associated with higher levels of aortic inflammation, but not CAC score or MFR. This suggests that galectin-3 may be a biomarker for an inflammatory and potentially reversible stage, but not a later (calcified) stage, of atherosclerosis in patients with RA.

Introduction

Cardiovascular disease (CVD) is the leading cause of excessive deaths in people with rheumatoid arthritis (RA) [1–3]. Current prediction tools for future CVD developed for the general population do not perform well in individuals with RA [4]. Biomarkers that augment prediction tools in identifying RA patients at high risk for CV events could enable better CV risk stratification and earlier intervention in RA patients.

Galectin-3 is a beta-galactoside-binding lectin found in both the intra- and extra-cellular environment in a wide range of tissues that has been implicated as both an indicator of inflammation as well as of CVD [5,6].

In a murine model of antigen-induced arthritis, galectin-3 knock out resulted in significantly reduced joint inflammation and bone erosion compared to wild-type (WT) mice [7]. In humans, galectin-3 levels were elevated in the sera and synovia of patients with RA compared with healthy controls or individuals with osteoarthritis (OA) [8], and were elevated in patients with undifferentiated arthritis who evolved into classifiable RA compared to those who did not [9].

In the general population, higher galectin-3 levels predicted incident heart failure (HF) [10] and serum galectin-3 level is FDA approved as a diagnostic marker for risk stratification and prognosis in HF evaluation

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[11]. Galectin-3 is also thought to be a key role in atherogenesis. In a murine model of atherosclerosis, there was increased expression of galectin-3 in intimal atherosclerotic plaques versus WT mice, and the atherosclerotic mice had higher serum levels of galectin-3 than WT mice [12]. Galectin-3 levels were detected in both early and advanced atherosclerotic lesions of carotid and limb arteries, localized by immunoperoxidase staining to macrophages and foam cells, suggesting a key role in early generation of atherogenesis- i.e., macrophage migration, lipid loading, and smooth cell proliferation [13].

Although similar studies in human arteries are lacking, galectin-3 levels were positively correlated with carotid intima-media thickness [14] and severity of coronary artery disease [15], and an elevated serum galectin-3 level has been shown to be an independent predictor of CVD mortality risk in individuals with coronary artery disease (CAD) [16].

Research exploring a potential role for galectin-3 in mediating CVD in patients with RA is limited to the work of Anyfanti et al. [17], who found an independent association between galectin-3 levels and cardiac output and systemic vascular resistance. However, the possible association of galectin-3 with joint inflammation [7–9] could confound its relationship with CVD in individuals with RA. We asked whether galectin-3 is an independent marker of subclinical vascular disease in an RA cohort extensively phenotyped for articular and subclinical CV disease.

We used coronary artery calcium (CAC) score, and 18Fluorodeoxyglucose positron emission-computed tomography (FDG PET/CT) of the ascending aorta, as structural and functional measures of atherosclerosis, respectively. FDG uptake in the vascular wall is thought to represent inflammation in atherosclerotic plaques [18], and is associated with future clinical CV events [19–20] but is reversible with treatment such as statins [21]. In contrast, CAC represents a more advanced (calcified) stage of atherosclerosis and does not appear to be reversible with statins [22]. Both measures were previously reported in our RA cohort without known clinical CVD [23]. Myocardial flow reserve (MFR) was used as a measure of dysfunction (impaired vasodilation) of the myocardial microvasculature and it also has been characterized in this same cohort [24]. We hypothesized that higher serum galectin-3 levels would be significantly associated with one or more of these vascular measures (higher CAC score, higher aortic inflammation, reduced MFR) in our cohort of RA patients without known clinical CVD, despite adjustments for levels of systemic and articular inflammation.

Materials and methods

Patients

The study sample consisted of 124 patients with RA enrolled in Rheumatoid Arthritis Study of the Myocardium (RHYTHM) who had a baseline serum galectin-3 level and all three cardiac measures (CAC, FDG PET/CT and MFR). RHYTHM has been described in detail previously [25–27]. Inclusion criteria were age ≥18 years and fulfillment of the 2010 American College of Rheumatology/European League Against Rheumatism criteria for RA [28]. Exclusion criteria included a prior CV event, defined as a self-reported physician-diagnosed myocardial infarction, HF, coronary artery revascularization, angioplasty, peripheral arterial disease or related procedures, pacemaker, or defibrillator devices. Other exclusion criteria included presence of active cancer and contraindication to pharmacologic study agents (i.e., regadenoson or adenosine). The study was in compliance with the Declaration of Helsinki and was approved by the Columbia University Institutional Review Board. All participants were provided written consent prior to participation.

Clinical covariables

Demographic and lifestyle characteristics were assessed by structured patient interviews using standardized health questionnaires. Current and past use of glucocorticoids and of disease-modifying antirheumatic drugs (DMARDs) was ascertained by patient interview and medical records, along with documentation of prescription and over-the-counter medications taken within the preceding two weeks verified by pill containers supplied by participants. Conventional synthetic DMARDs (csDMARD) included methotrexate, sulfasalazine, hydroxychloroquine, and leflunomide. Biologic DMARDs (bDMARDs) included adalimumab, etanercept, infliximab, certolizumab, golimumab, tocilizumab, abatacept, anakinra, and rituximab. Body mass index (BMI) was calculated as patient weight (in kilograms) divided by height (in square meters). Resting blood pressure (BP) was measured 3 times, with the mean of the last 2 measurements used. Hypertension (HTN) was defined as a systolic BP of ≥140 mm Hg, diastolic BP of ≥90 mm Hg, or use of antihypertensive medications. Diabetes was defined as a fasting serum glucose level of ≥126 mg/dl or use of antidiabetic medications. RA disease duration was measured in years from the date of physician diagnosis. Disease activity was calculated based on the 28 joint count using the Clinical Disease Activity Index (CDAI), and the Disease Activity Score using C-reactive protein (DAS28-CRP) formulas [29–31]. The Health Assessment Questionnaire (HAQ) was used to assess functional impairment [32].

Laboratory measurements

Phlebotomy was performed after an overnight fast. Serum and plasma were separated by centrifugation and stored at −80 °C. All samples were measured in the Biomarkers Core Laboratory of the Columbia University Irving Institute for Clinical and Translation Research. Galectin-3 levels were measured by ELISA, reported in ng/mL (Quantikine®ELISA kit, R&D Systems, Intra-Assay Precision Coefficient of Variations 3.8 %, Inter-Assay Precision Coefficient of Variations 6.3 %). Anti-cyclic citrullinated peptide antibody (anti-CCP) was measured by ELISA (Quanta Lite CCP3 IgG kit, Inova Diagnostics), with seropositivity defined as ≥60 units. Rheumatoid factor (RF) was measured by ELISA (IBL America), with seropositivity defined as ≥40 units.

Imaging

^{18}Fluorodeoxyglucose [FDG] positron emission-computed tomography [FDG PET/CT]:

Aortic inflammation was assessed and quantified via FDG-PET/CT scan using reproducible and validated methods [18,33]. The scan was performed on the same visit as the phlebotomy visit, after 18 h of a carbohydrate-free diet and an overnight fast. Analyses were restricted to the ascending aorta. The area of interest was defined as the region of the aorta beginning 1 cm above the origin of the left main coronary artery and ending at the aortic arch in trans-axial view. Ascending aortic inflammation was determined by FDG uptake in the arterial wall, calculated as the standardized uptake value (SUV). The outcome measure used in our multivariable models was maximal SUV of the most diseased segment (SUV MDS max) as defined previously [21,33].

Coronary artery calcium (CAC). Subclinical atherosclerosis was assessed using CAC. CAC was quantified by the Agatston method using the cardiac CT images obtained for attenuation correction and anatomic coregistration with the PET scan [34].

Myocardial flow reserve (MFR). Microvascular dysfunction was assessed by calculating myocardial flow reserve (MFR). This was done by measuring myocardial blood flow (MBF) before (rest) and after vasodilation (stress) using PET/CT with N-13 ammonia as a perfusion tracer, as described previously [24]. The ratio of the MBF measured at maximal vasodilation over the MBF at rest is referred to as MFR, which is a measure of the vasodilatory reserve of the myocardium. Reduced MFR in the absence of flow-limiting CAD is believed to reflect dysfunction in the myocardial microvasculature. Detailed methods for assessing MFR in this cohort have been published previously [24].

Statistical analysis

All analyses were performed on baseline data. Galectin levels and CAC were measured on all 124 patients enrolled, while aortic FDG uptake and MFR were measured in 89 and 80, respectively, at baseline. Summary statistics for variables were calculated. Means and standard deviations for normally distributed, and medians and interquartile ranges for non-normally distributed variables, were calculated. For categorical variables, counts and percentages were calculated. Univariable linear regression models were used to explore the associations of RA patient characteristics, treatment, atherosclerosis risk factors, and measures of cardiac and arterial structure and function, with log transformed galectin-3 levels. To assess the independent association of galectin-3 level and aortic inflammation, variables that were associated with the log transformed galectin-3 level in the univariable models at a significance level of $p < 0.30$ were carried into the extended multivariate linear regression models. The extended multivariate model was reduced further to a limited multivariable linear regression model by including all the variables from the extended multivariable model with p values <0.30, to account for the possibility of residual confounding. We also performed multivariable analyses stratified by age, gender, race, treatment type, CCP or RF positivity, disease activity, smoking status, and CAC. In all tests, a 2-sided alpha level of 0.05 was considered statistically significant. Serum galectin level was modeled as a continuous variable, but split into tertiles in Tables 1 and 2 for the summary statistics, for easier visualization and interpretation. All statistical calculations were performed using Stata software.

Results

Patient characteristics

Baseline characteristics of the 124 RA patients are summarized in Tables 1 and 2. The mean age was 57 years. The majority were women (82 %), of diverse race and ethnicity (45 % Hispanic), and overweight on average (mean BMI of 29). Median RA duration was 6.8 years. Seventy-five percent were RF or CCP positive. The majority of patients had low to moderate disease activity, with median DAS-CRP of 3.91 and CDAI of 16. One third of patients were currently on prednisone, with median dose of 5 mg daily. The majority (79 %) of patients were prescribed a csDMARD and 28 % of those were also prescribed a bDMARD. Ten percent of patients were on a bDMARD only, and 11 % of patients were not on any DMARD. Forty-three percent were current or former smokers, 38 % had hypertension, 11 % had diabetes, 15 % were on a statin, and 10 % were on aspirin. The majority (66 %) of participants had a CAC score of zero with only 10 % of patients having a CAC score ≥ 300. The median ascending aorta SUV MDS max was 2.59. The mean MFR was 2.86. Median galectin-3 level was 8.54 ng/mL.

Univariable associations of patient characteristics and galectin-3 level

Baseline characteristics are summarized in Tables 1 and 2. In univariable analyses, older age, race, longer RA disease duration, current prednisone use, type of treatment, higher triglyceride level, presence of diabetes, and higher levels of ascending aorta inflammation as measured by SUV were all associated with increased galectin-3 levels. Measures of disease activity (DAS28-CRP, CDAI) and systemic inflammation (IL-6

Table 1
Baseline characteristics of RA cohort (demographics, RA characteristics and current.

	All patients ($n = 124$)	Patients in 1st tertile of galectin-3 level [3.3–7.33 ng/mL] ($n = 42$)	Patients in 2nd tertile of galectin-3 level [7.44–9.51 ng/mL} ($n = 42$)	Patients in 3rd tertile of galectin-3 level [9.54- 38.44 ng/mL] ($n = 42$)	p value [†]
Demographics					
Mean Age – years	56.5 ± 12.26.	51.76 ± 13.38	55.07 ± 11.43	57.10 ± 11.55	0.008
Female – no. (%)	101 (81.45)	31 (73.81)	35 (85.37)	35 (85.37)	0.745
Race/Ethnicity					
Non-Hispanic White – no. (%)	43 (35.25)	18 (43.90)	15 (37.50)	10 (24.39)	ref
Non-Hispanic Black – no. (%)	19 (15.57)	4 (9.76)	7 (17.50)	8 (19.51)	0.344
Hispanic – no. (%)	55 (45.08)	15 (36.59)	18 (45.00)	22 (53.66)	0.039
Other – no. (%)	5 (4.10)	4 (9.76)	0	1 (2.44)	0.904
RA Characteristics					
Median RA duration – years	6.8 (2–14.2)	6.9 (2–13)	6.6 (1.5–17.6)	6.9 (2.8–16.6)	0.013
RF positive – no. (%)	68 (54)	22 (52.38)	26 (63.41)	20 (48.78)	0.774
Anti-CCP positive – no. (%)	84 (67.74)	28 (66.67)	31 (75.61)	25 (60.98)	0.566
Nodules Present – no. (%)	10 (8.13)	3 (7.14)	1 (2.50)	1 (2.44)	0.425
Median IL-6 pg/mL[#]	2.30 (1.38–7.53)	1.96 (1.10–3.73).	2.48 (1.24–8.71)	2.81 (1.88–8.24)	0.096
Median CRP mg/L[#]	2.48 (1.09–6.67)	2.24 (1.09–6.6)	2.09 (0.77–6.06)	3.26 (1.21–8.32)	0.617
Median DAS28-CRP	3.91 (2.98–4.69)	3.68 (2.63–4.44)	3.68 (2.81–4.70)	4.30 (3.35–4.84)	0.122
Median CDAI	16.4 (8.6–28.1)	15.5 (6.5–24.9)	12.7 (8.1–31.3)	21.8 (12.1–32.1)	0.075
Median HAQ		0.94 (0.38–1.5)	1.34 (0.88–1.88)	1.25 (0.63–2.0)	0.058
Current Treatment With:					
Prednisone use– no. (%)	41 (33.06)	9 (21.43)	15 (36.59)	17 (41.46)	0.038
Median prednisone dose (mg)	5 (4–10)	5 (4–7.5)	5 (2.5–10)	5 (4.5–7.5)	0.406
Conventional DMARD with no biologic – no. (%)	63 (50.81)	23 (54.76)	20 (48.78)	20 (48.78)	ref
Conventional DMARD with biologic – no. (%)	35 (28.23)	13 (30.95)	13 (31.71)	9 (21.95)	0.748
Biologic only – no. (%)	12 (9.68)	2 (4.76)	2 (4.88)	8 (19.51)	0.006
No DMARD – no. (%)	14 (11.29)	4 (9.42)	6 (14.63)	4 (9.76)	0.932

RA = rheumatoid arthritis; RF = rheumatoid factor; anti-CCP = anti–cyclic citrullinated peptide; IL-6 = interleukin-6; CRP = C-reactive protein level; DAS28-CRP = Disease Activity Score in 28 joints using the CRP level; HAQ = Health Assessment Questionnaire; DMARD = disease-modifying antirheumatic drug.
Means reported with (Standard Deviation); Medians reported with (Interquartile range).

[†] p value of univariable linear regression association between patient characteristics against the log transformed value of galectin-3 level. Values <0.05 represent statistical significance.

[#] Values log-transformed when included in univariable regression model.

Table 2
Baseline characteristics of RA cohort (cardiovascular risk factors and outcome.

	All patients ($n = 124$)	Patients in 1st tertile of galectin-3 level [3.3–7.33 ng/mL] ($n = 42$)	Patients in 2nd tertile of galectin-3 level [7.44–9.51 ng/mL} ($n = 42$)	Patients in 3rd tertile of galectin-3 level [9.54- 38.44 ng/mL] ($n = 42$)	*p* value [†]
Cardiovascular risk factors					
Ever smoker – no. (%)	53 (43.09)	16 (38.10)	18 (45.00)	19 (46.34)	0.193
Current smoker – no. (%)	15 (12.20)	5 (11.90)	6 (15.00)	4 (9.76)	0.775
HTN – no. (%)	48 (38.71)	9 (21.43)	22 (53.66)	17 (41.46)	0.119
Mean LDL mg/dL	110.35 ± 32.36	112.62 ± 26.07	115.68 ± 35.75	102.70 ± 33.92	0.200
Mean HDL mg/dL	59.9 ± 19.15	61.29 ± 22.40	59.24 ± 18.51	57.29 ± 16.20	0.179
Median Triglycerides mg/d [#]	91.5 (75–132)	84.5 (74–112)	96 (77–138)	90 (75–140)	0.019
Diabetes – no. (%)	14 (11.29)	1 (2.38)	8 (19.51)	5 (12.20)	0.033
Statin use – no. (%)	19 (15.32)	5 (11.90)	6 (14.63)	8 (19.51)	0.259
Aspirin use – no. (%)	12 (9.68)	2 (4.76)	8 (19.51)	2 (4.88)	0.99
Mean BMI kg/m2	28.56 ± 5.79	28.67 ± 5.47	26.92 ± 4.98	30.05 ± 6.53	0.194
Median Galectin level ng/mL[#]	8.54 (6.64–10.03)	5.87 (4.53–6.76)	8.55 (7.9–9.05)	13.4 (10.04–17.5)	n/a
Measures of atherosclerosis and vascular inflammation					
CAC Score Median CAC score[#]	0 (0–34)	0 (0–28)	0 (0–9.5)	0 (0–139)	0.121
CAC score of zero – no. (%)	80 (65.57)	29 (72.50)	28 (70.00)	22 (53.66)	0.093
CAC score ≥ 100 – no. (%)	23 (19.01)	5 (12.50)	6 (15.00)	12 (29.27)	0.057
CAC score ≥ 300 – no. (%)	12 (9.92)	3 (7.50)	3 (7.50)	6 (14.63)	0.345
Standardized Uptake Values (SUV) at the Aorta [‡]					
Median SUV max[#]	2.43 (2.15–2.77)	2.30 (1.97–2.83)	2.52 (2.18–2.75)	2.54 (2.32–2.73)	0.007
Median SUV MDS max[#]			2.62 (2.36–2.97)	2.82 (2.4–3.32)	0.007
Median SUV mean[#]	1.83 (1.6–2.09)	1.68 (1.49–2.01)	1.87 (1.64–2.1)	1.93 (1.71–2.19)	0.003
Median SUV MDS mean[#]	1.91 (1.64–2.19)	1.73 (1.5–2.04)	1.92 (1.71–2.18)	2.06 (1.74–2.29)	0.004
Mean MFR[*]	2.86 ± 0.73	2.98 ± 0.85	2.81 ± 0.66		0.749

HTN: hypertension; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; BMI = body.
mass index; CAC = coronary artery calcification; SUV = standardized uptake value in units; max = maximum; MDS = most diseased segment; MFR= myocardial flow reserve.
Means reported with (Standard Deviation); Medians reported with (Interquartile range).
[†] *p* value s of univariable linear regression association between patient characteristics against the log transformed value of galectin-3 level. Values <0.05 represent statistical significance.
[#] Values log-transformed when included in univariable regression model.
[‡] $n = 89$.
[*] $n = 80$.

and CRP) were not associated with galectin-3 level. CAC score and MFR were not associated with galectin-3 level.

Multivariable association of galectin-3 level with ascending aortic inflammation

The association between galectin-3 level and arterial inflammation, as measured by SUV MDS max, was further explored using multivariable models. In our final reduced multivariable model, higher galectin-3 level remained significantly associated with greater arterial inflammation as measured by SUV MDS max in the ascending aorta, after adjusting for treatment type and BMI (Table 3).

Interestingly, the association of galectin-3 level with aortic SUV MDS max differed by gender. Each log unit higher galectin-3 level was associated with a 0.22 unit higher aortic SUV MDS max in women ($p = 0.001$), but was but was not significantly associated in men ($p = 0.720$) (p for interaction = 0.030) (Fig. 1). The association of galectin-3 level with aortic SUV MDS max also differed by race. Each log unit higher galectin-3 level was associated with a 0.27 unit higher aortic SUV MDS max in Non-Hispanic Whites ($p = 0.004$), but was not significantly associated in Non-Whites ($p = 0.254$) (p for interaction = 0.089) (Fig. 2). The association of galectin-3 level with aortic SUV MDS max also differed by treatment type. Higher galectin-3 level was significantly associated with higher aortic SUV MDS max in patients who were not receiving biologics (B coefficient= 0.2955, *p* value = <0.001), but was not significantly associated in patients receiving biologics (B coefficient = −0.0034, *p* value = 0.961) (*p*-value for interaction = 0.004). The association of galectin-3 level with aortic SUV MDS max did not differ as a function of age, CCP or RF positivity, DAS28-CRP, smoking status, or presence of CAC (data not shown).

Discussion

Previous research suggests a potential association between galectin-3 level and joint inflammation in patients with RA, while research in the general population supports an association between galectin-3 level and various outcome measures of CVD. The association of galectin-3 with disease activity could hence confound the relationship between galectin-3 and CVD in individuals with RA and thus limit its usefulness as a marker of CVD in RA. Research exploring serum galectin-3 level and measures of CVD in RA cohorts is limited. In our cohort of RA patients without known clinical CVD, we found that higher serum galectin-3 levels were associated with higher levels of aortic inflammation but not with articular or systemic inflammation, a distinguishing feature from the few prior RA studies. Moreover, galectin-3 level was not associated with advanced atherosclerosis, as measured by CAC score, or with microvascular dysfunction, as measured by MFR. These results suggest that galectin-3 may be a biomarker for an earlier, inflammatory and potentially reversible stage, but not a later (calcified) stage, of atherosclerosis in patients with RA.

Although early studies in mice and in humans suggested a positive signal between galectin-3 levels and joint inflammation [7–9], galectin-3 was not associated with any measures of RA disease activity or systemic inflammation in our cohort. Oshima et al., one of the first groups to study galectin-3 in RA, found a positive association between

Table 3
Multivariable associations of RA patient characteristics and aortic SUV MDS max.

Log Aortic SUV MDS max	Extended multivariable model $n = 89$		Reduced multivariable model $n = 89$	
	ß Coefficient	*p*-value	ß Coefficient	*p*-value
Log galectin-3 level per ng/mL	0.1856	0.007	0.1786	0.002
Age – years	0.0025	0.308		
Race/Ethnicity				
Non-Hispanic White	ref	ref		
Non-Hispanic Black	−0.0027	0.974		
Hispanic	0.0127	0.844		
Other	0.1047	0.445		
RA duration – years	−0.0009	0.717		
Log IL-6 level per pg/mL	−0.0273	0.523		
DAS28-CRP	0.0036	0.893		
HAQ	0.0022	0.955		
Current Prednisone use	−0.0521	0.409		
Type of Treatment Receiving				
Conventional DMARD with no biologic	ref	ref	ref	ref
Conventional DMARD and biologic	0.1287	0.039	0.1048	0.183
Biologic only	−0.0712	0.434	−0.1001	0.051
No DMARD	−0.0034	0.968	−0.0293	0.693
Ever smoker	0.0560	0.322		
HTN	0.0026	0.966		
Diabetes	−0.0509	0.633		
Statin use	−0.0477	0.533		
BMI kg/m^2	0.0115	0.015	0.2411	0.123
Prob >*F*	0.0918		0.0004	
Adjusted R2	0.1049		0.1876	

The extended multivariable model includes variables from Table 1 with a univariable association *p* value <0.30. Statin use was selected as a surrogate for HDL, LDL and triglyceride levels. The reduced multivariable model includes all of the variables from the extended model with *p* value s <0.3. See Tables 1 and 2 for abbreviations.

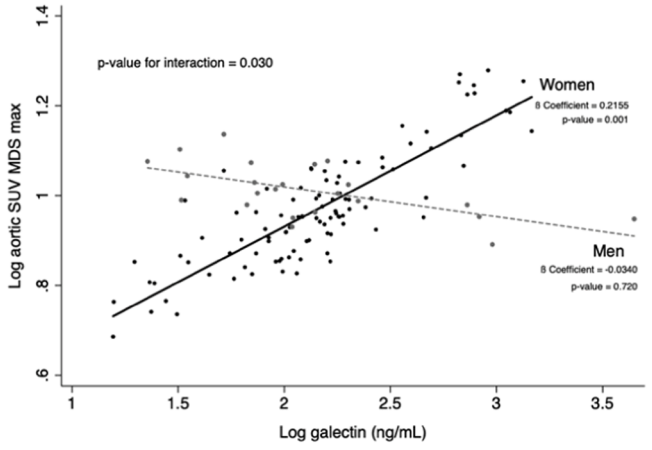

Fig. 1. Multivariable association of galectin-3 level with ascending aorta inflammation stratified by gender.

serum galectin-3 level and CRP, but the treatment status was not reported in the paper, and this study was published almost two decades ago in a small sample size, when biologic DMARD treatment options were limited, [8]. Congruent to our findings, Anyfanti et al. did not find any association between galectin-3 level and ESR, CRP, or DAS score in their cohort, in which all patients were receiving DMARD treatment [17]. Mendez-Heurgo et al. also did not find an association between galectin-3 levels and measures of RA disease activity including ESR, DAS-28, or VAS in their cohort, and their patients were treated with at least one DMARD [35].

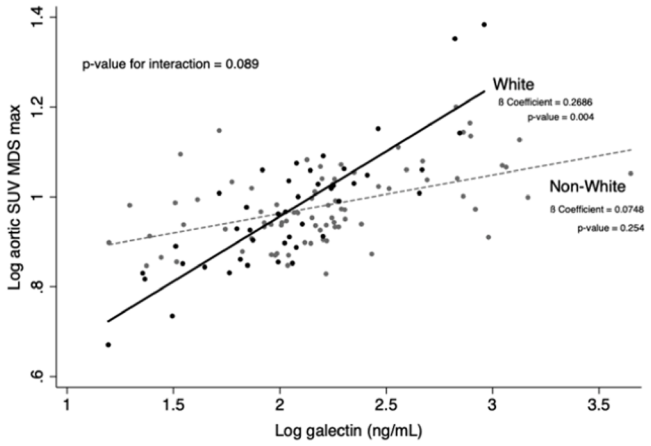

Fig. 2. Multivariable association of galectin-3 level with ascending aorta inflammation stratified by race.

Higher FDG uptake in the aortic vessel wall has been shown to correlate with macrophage infiltrates and is a risk factor for plaque rupture and future CV events [19,20]. Moreover, statin therapy and RA disease modifying agents have been shown to reduce vascular inflammation as measured by FDG/PET CT [21,36].

The higher serum concentrations of galectin-3, which correlated with higher aortic FDG uptake in our cohort, may reflect active arterial inflammation and plaque instability. In a murine model of atherosclerosis, higher intra-plaque galectin-3 level correlated with higher degrees of active inflammation, measured by macrophage content in histopathologic samples, and treatment with atorvastatin decreased the intraplaque galectin-3 concentration [12]. In clinical studies of patients with angiography proven CAD, serum galectin-3 levels were significantly higher in patients with unstable angina and acute MI compared with those with stable angina [37].

In contrast, CAC score appears to correlate with more stable plaque [38,39]. This could be a potential reason why no statistically significant relationship was found between galectin-3 level and CAC score in our RA cohort. Although in the RHYTHM cohort we did not measure carotid intimal thickness (cIMT), another measure of atherosclerosis, Anyfanti et al. reported a positive association between galectin-3 level and cIMT in RA patients [17].

Interestingly, the independent relationship between higher galectin-3 level and higher levels of aortic inflammation differed based on sex, race, and treatment type, but not on age, seropositivity, DAS28-CRP, smoking status, or presence of CAC. When the cohort was stratified by sex, the association was only significant in women. This is similar to a non-RA study in which galectin-3 level was significantly associated with incident HF only in women, not men [40]. We acknowledge the possibility of being unable to detect significant associations in men, however, due to the small number enrolled in RHYTHM ($n = 23$). Race also influenced the relationship, as the association between galectin-3 and aortic inflammation was significant only in whites compared to non-whites. This finding has also been seen in HF, where in a cohort of 1375 white and 434 black individuals, galectin-3 level was independently associated with composite HF or death in whites, but not in blacks [41].

Treatment type also appears to play a role. In our cohort, higher galectin-3 level was significantly associated with higher aortic inflammation in patients who were not on biologics, but not in patients who were on biologics. The majority of biologics prescribed to the RHYTHM patients were TNF-inhibitors. Previous studies showed that galectin-3′s relationship with TNF is complex, and dependent on tissue type and disease. For example, galectin-3 induced higher levels of TNF in synovial fibroblasts than in skin fibroblasts [42]; however, in a model of viral induced hepatitis, galectin-3 attenuated TNF-mediated death of

hepatocytes [43]. When an RA patient is being treated with a biologic, the complex interaction between galectin-3 and other cytokines is disrupted and may explain why no relationship was found in this subgroup.

In addition to investigating the association between galectin-3 level and arterial inflammation and CAC as outcome measures of atherosclerosis, we also examined the association between galectin-3 level and MFR, where reduced MFR is thought to reflect microvasculature dysfunction (reduced vasodilation) of the myocardium. We found no association between serum galectin-3 level and MFR in our RA cohort; however, this is the first study to ever look at the association between galectin-3 level and MFR, and more research is needed in this area to draw any conclusions.

Additionally, comparing galectin-3 levels from our study across other non-RA studies remains challenging due to wide variabilities in assay used as well as cohort characteristics . One large general population study of over 3000 patients reported serum galectin-3 levels (first quartile) between 3.9–11.1 ng.ml for men and 5.0 to 12.0 ng/ml for women, which would fit most of our patients in this study [10].

Strengths of our study include a unique cohort with extensive structural and functional CV phenotyping and racial and ethnic diversity, as well as a range of disease activity, disease duration and treatments, making it reflective of a real-world RA population. Limitations of our study include its cross-sectional nature, a relatively small number of patients with arterial FDG uptake and MFR measurements, and the lack of a non-RA control group.

Conclusions

In conclusion, our results suggest that serum galectin-3 level may serve as a biomarker for an early, active, unstable, and potentially reversible inflammatory stage of atherosclerosis, but not a later stage of atherosclerosis, in patients with RA. It is clear from the growing body of literature that galectin-3′s role in both atherogenesis and RA is complex and deserves additional study.

Clinical significance

Galectin-3, a beta-galactoside-binding lectin, is a marker of cardiovascular disease (CVD) and heart failure (HF). The role of Galectin-3 has yet been explored in systemic inflammation or joint disease. In our cohort of RA patients without clinical CVD, high levels of galectin-3 were independently associated with higher levels of aortic inflammation.

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Declaration of competing interest

The authors declare there is no conflict of interests.

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