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ORIGINAL RESEARCH

Changes in serum cholesterol loading capacity are linked to coronary atherosclerosis progression in rheumatoid arthritis

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ABSTRACT

Objective Excess cholesterol loading on arterial macrophages is linked to foam cell formation, atherosclerosis and cardiovascular risk in rheumatoid arthritis (RA). However, the effect of changes in cholesterol loading on coronary plaque trajectory and the impact of RA therapies on this relationship are unknown. We investigated the association between variations in cholesterol loading capacity (CLC) over time and atherosclerosis progression.

Methods In a prospective observational cohort study, coronary CT angiography evaluated atherosclerosis (non-calcified, partially calcified or fully calcified plaques and coronary artery calcium (CAC) score) in 100 patients with RA without cardiovascular disease at baseline and 6.9±0.4 years later. The presence of ≥5 plaques and lesions rendering >50% stenosis was considered an extensive and obstructive disease, respectively. Serum CLC was measured on human THP-1 monocyte-derived macrophages with a fluorometric assay.

Results Mean CLC change (follow-up CLC–baseline CLC) was 1.54 (SD 3.69) µg cholesterol/mg protein. In models adjusting for atherosclerotic cardiovascular disease risk score, baseline plaque and other relevant covariates, CLC change (per SD unit increase) is associated with a higher likelihood of progression of non-calcified (OR 2.55, 95% CI 1.22 to 5.35), fully calcified plaque (OR 3.10, 95% CI 1.67 to 5.76), CAC (OR 1.80, 95% CI 1.18 to 2.74) and new extensive or obstructive disease (OR 2.43, 95% CI 1.11 to 5.34). Exposure to prednisone unfavourably influenced, while biologics and statins favourably affected the relationship between CLC change and atherosclerosis progression (all p-for-interactions ≤0.048).

Conclusion CLC change is associated with atherosclerosis progression in a dose-dependent manner, including lipid-rich non-calcified plaques and extensive or obstructive disease that yield the greatest cardiovascular risk.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Serum cholesterol loading capacity on macrophages has been linked to atherosclerosis and cardiovascular risk in rheumatoid arthritis.

WHAT THIS STUDY ADDS

⇒ Baseline to follow-up change in cholesterol loading capacity associated with atherosclerosis progression in a dose-dependent manner.

⇒ The relationship between cholesterol loading capacity change and atherosclerosis progression was unfavourably influenced by prednisone exposure and favourably affected biologics and statins.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Therapies targeting cholesterol loading in macrophage foam cells could complement conventional therapies to reduce cardiovascular risk.

INTRODUCTION

Patients with rheumatoid arthritis (RA) exhibit greater coronary atherosclerosis burden, plaque vulnerability, progression and long-term cardiovascular risk compared with age- and gender-matched controls.[1–3] Excess cholesterol accumulation in arterial wall macrophages and foam cell formation are critical steps in the development of atherosclerotic plaque.[4] Cholesterol transport in the serum and loading on cells is performed primarily by low-density lipoprotein (LDL). Hence, the capacity of serum to supply cholesterol to cells is referred to as serum cholesterol loading capacity (CLC). This is a strictly regulated process, with native LDL onloading cholesterol to cells via the LDL receptor. However, in the context of RA-related inflammation, structural modifications of LDL, such as oxidation, promote excess cholesterol loading on arterial macrophages through alternate and unregulated pathways that significantly enhance foam cell formation.[5–7]

We recently showed that oxidised LDL (oxLDL) directly influenced cholesterol loading on macrophages independently of inflammation in patients with rheumatoid factor and anticitrullinated protein antibody-positive RA.[8] Moreover, anti-oxLDL IgG antibodies as well as proprotein convertase subtilisin kexin type-9 mediated the effects of oxLDL on macrophage cholesterol loading depending on seropositivity and grade of inflammation.[8] Consistent with our findings, plasma from patients with RA yielded significantly greater transformation of macrophages to foam cells compared with control plasma.[9] This transition was linked to the upregulation of membrane scavenger receptors facilitating cholesterol uptake and a reciprocal downregulation of transporter proteins that facilitate cholesterol efflux out of plaque macrophages.[9] Importantly, CLC is associated with cardiovascular risk, atherosclerosis burden and plaque vulnerability, particularly in biologic disease-modifying antirheumatic drug (bDMARD) non-users in RA.[10]

Coronary atherosclerosis progression is linked to accelerated cardiovascular risk independently of baseline plaque burden,[11 12] whereas stabilisation is associated with decreased risk of future events in general patients.[13] RA-related therapies, including corticosteroids, bDMARDs and lipid-lowering agents, influence atherosclerotic plaque progression and cardiovascular risk in opposing ways.[2 14–16] Yet, it is currently unknown whether variations in macrophage cholesterol loading over time are linked to changes in coronary atherosclerosis burden in RA. In the present study, we evaluated whether changes in serum CLC are associated with plaque progression in RA and if RA-specific or lipid-lowering therapies influence this relationship.

METHODS

Patient recruitment

We evaluated 100 patients enrolled in our formerly reported Prospective Evaluation of Latent Coronary Atherosclerosis in Rheumatoid Arthritis cohort[1] with non-invasive coronary atherosclerosis assessments via coronary CT angiography (CCTA) at baseline and 6.9±0.4 years later. The original cohort encompassed 150 patients with RA recruited on a first-come, first-served basis between March 2010 and March 2011. Subjects were aged between 18 and 75 years, fulfilled 2010 classification criteria for RA, had serum available for CLC measurement, had no prior atherosclerosis imaging and had no prior diagnosis of cardiovascular disease, including acute coronary syndrome, stable angina, stroke, transient ischaemic attack, peripheral arterial disease, revascularisation or heart failure. Participants with overlapping autoimmune syndromes (except Sjogren's), weight >147.7 kg (scanner bed capacity), malignancy within 5 years, chronic or active infections, glomerular filtration rate <60 mL/min or iodine allergy were excluded. The study was approved by the local institutional review board and participants provided written informed consent according to the Declaration of Helsinki.

Coronary CT angiography

Baseline assessments were carried out between March 2010 and March 2011 and surveillance assessments occurred between March 2017 and March 2018 on a 256-multidetector row scanner. Detailed descriptions of image acquisition, processing protocols and interobserver/intraobserver grading reproducibility for our centre have been previously reported.[14] The coronary artery calcium (CAC) score was measured in a non-contrast scan.[17] Plaque presence and burden were evaluated on contrast-enhanced scans using a standardised 17-segment American Heart Association model.[18] Both baseline and follow-up studies for each participant were reviewed concurrently and in random order by an experienced, blinded reader (MJB). Coalignment of coronary segments using fixed anatomic landmarks as fiducial points allowed for longitudinal comparisons of change in atherosclerosis burden. Numbers of coronary segments with plaque (0–17) per patient were reported. The stenotic severity of individual lesions was evaluated as previously detailed.[1] The presence of ≥5 coronary plaques in a patient and lesions rendering greater than 50% luminal stenosis were considered extensive and obstructive disease, respectively; both parameters have been linked to very high cardiovascular risk.[19 20] Plaque composition was reported as non-calcified, partially calcified and fully calcified as previously described.[1]

Laboratory evaluations

Rheumatoid factor and anticitrullinated protein antibody status at diagnosis were collected through the electronic medical record. Blood samples for complete blood counts, metabolic panel, erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) were collected during baseline and follow-up coronary atherosclerosis imaging and upon every interim clinic visit. Fasting serum lipids were measured on the day of baseline and follow-up scans and according to European Alliance of Associations for Rheumatology (EULAR) recommendations in between.[21] Serum for additional biomarker studies was stored at −80°C as previously described.[1]

Serum CLC

Serum CLC was measured on samples collected during baseline and follow-up coronary atherosclerosis assessments. CLC was quantified on human THP-1 monocyte-derived macrophages using a fluorometric assay as previously detailed.[22] Briefly, THP-1 cells were cultured with 100 ng/mL phorbol 12-myristate 13-acetate (PMA) for 72 hours to allow differentiation into macrophages. The cells were subsequently incubated with individual sera from patients with RA at 5% dilution for 24 hours. Next, cells were washed and lysed, and cholesterol content was measured fluorometrically in cell lysates. CLC was expressed as micrograms of cholesterol per milligram

of protein. CLC change was calculated as the difference between follow-up and baseline CLC ($CLC_{follow\text{-}up}$–$CLC_{baseline}$).

Covariates and outcomes

The 10-year atherosclerotic cardiovascular disease (ASCVD) risk score based on pooled cohort equations was estimated for all participants at baseline. Obesity was defined as body mass index ≥30 kg/m^2. Disease activity was computed based on a 28-joint count examination for tenderness and swelling and CRP (DAS28-CRP). Medications, including methotrexate, other conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), bDMARD, prednisone and statin use, were recorded on each visit and cross-referenced against pharmacy records. Time-averaged CRP for each patient was calculated by summing the mean CRP values between consecutive measurements multiplied by the time interval between consecutive measurements and then dividing by the patient's total follow-up time.[23]

The dependent variables were six baselines used to follow up plaque change outcomes. Total plaque increase was the number of segments with any new plaque. For the three plaque subtypes, progression was defined as ≥1 new segment with non-calcified, partially calcified or fully calcified plaque. CAC progression was defined as a change in the square root-transformed CAC score ≥2.5.[24] For extensive or obstructive plaque, progression was defined as new onset extensive plaque (≥5 segments) and/or ≥1 new segment with obstructive plaque.

Statistical analysis

Continuous variables were presented as means with SD and categorical variables as numbers with percentages. Non-normally distributed variables were natural logarithm transformed. Unadjusted differences in CLC change between patients with and without plaque progression were evaluated with t-tests. The effect of CLC change was tested using robust logistic regression for binary plaque progression outcomes (non-calcified, partially calcified, fully calcified, extensive or obstructive and CAC) and robust negative binomial regression for the count of the number of segments with any new plaque. To explore the interaction of CLC change with exposure to prednisone, bDMARDs and statins during follow-up, medication exposure and its interaction with CLC change were added to the plaque change models. Medication exposure was defined as ever use of the index agent throughout the study duration. Never users were considered unexposed. All models adjusted for ASCVD risk score, baseline plaque burden and covariates with p<0.05 in the multivariable model. There were no missing data on evaluated variables. Analyses were performed in Stata V.15.0 and two-tailed p values <0.05 were considered significant.

RESULTS

Of 150 patients in our originally described cohort with baseline coronary atherosclerosis evaluation, 102 underwent surveillance plaque evaluation 6.9±0.4 years later. Of the 48 that did not, four had no follow-up beyond the baseline visit, six migrated, two died and 36 declined to participate in a follow-up evaluation. Baseline characteristics of patients stratified by follow-up CCTA are shown in online supplemental table S1. Patients without follow-up CCTA were older, had more tender joints and had a greater cardiovascular risk score. However, those differences became insignificant after adjusting for age. Of the 102 patients with follow-up plaque evaluation, 100 had both baseline and follow-up blood samples for CLC evaluation and were therefore included in the present study.

The sample was generally middle-aged females with established, seropositive and erosive disease. Patient characteristics at baseline and follow-up are summarised in table 1. All patients received csDMARDs (78% methotrexate) and 63/100 (63%) additionally received tumour necrosis factor-alpha inhibitor (TNFi) bDMARDs at baseline. Throughout follow-up, 75 (75%) patients were exposed to bDMARDs (including non-TNFi), 47 (47%) to prednisone and 53 (53%) to statins. At baseline, 69/100 (69%) patients had any plaque presence (a total of 189 lesions), 30/100 (30%) had CAC >0 and 16/100 (16%) had extensive or obstructive disease. On follow-up assessment 6.9±0.4 years later, 69 (69%) patients had any plaque (a total of 248 lesions). In 10 patients with only non-calcified plaques at baseline, these plaques regressed. Another 10 patients without plaque at baseline developed new plaques. 14 patients (14%) had non-calcified plaque progression, 25 (25%) had partially calcified plaque progression, 35 (35%) had fully calcified plaque progression, 37 (37%) had CAC progression, and 15 (15%) had new extensive or obstructive plaque at follow-up.

Change in serum CLC over time

CLC decreased in 68 (68%) patients and the mean±SD CLC change was 1.54±3.69 µg cholesterol/mg protein (range −11.35 to +6.42). CLC change was associated with time-averaged ESR (Pearson's r=0.200, p=0.048) but not time-averaged CRP (Pearson's r=−0.029, p=0.773). There were no differences in CLC change among patients exposed to prednisone, bDMARDs and statins at any time and those who were not (online supplemental table S2). CLC change was greater in patients with ≥1 new non-calcified plaque (0.50±3.37 µg cholesterol/mg protein) compared with those with none (−1.87±3.64 µg cholesterol/mg protein, p=0.016). This was also true for patients with ≥1 new calcified plaque (−0.35±3.64 µg cholesterol/mg protein) versus without (−2.17±3.58 µg cholesterol/mg protein, p=0.018), CAC progression (−0.59±3.48 µg cholesterol/mg protein) versus not (−2.09±3.72 µg cholesterol/mg protein, p=0.045) and new extensive or obstructive plaque versus without (0.04±3.32 vs

Table 1 Patient characteristics at baseline and follow-up (n=100)

	Baseline		Follow-up	
Age (years)	51.47	±10.30	58.42	±10.23
Male, no. (%)	14	(14%)	—	
RA-related parameters				
RA duration (years)	10.09	± 7.08	17.02	± 7.07
Age at diagnosis (years)	41.41	± 10.39	—	
RF positive, no. (%)	86	(86%)	—	
ACPA positive, no. (%)	85	(85%)	—	
Erosions, no. (%)	64	(64%)	76	(76%)
C reactive protein (mg/dL)	0.85	±1.28	0.81	±1.29
DAS28-CRP	1.95	±0.82	2.21	±1.11
Cardiovascular risk factors				
CLC (µg cholesterol/mg protein)	12.61	±2.90	11.07	±1.86
Total cholesterol (mg/dL)	165.93	±34.61	181.81	±39.58
LDL-c (mg/dL)	93.95	±28.11	101.62	±31.54
HDL-c (mg/dL)	50.85	±12.98	52.47	±15.68
Systolic blood pressure (mm Hg)	126.17	±15.21	132.49	±18.68
Diastolic blood pressure (mm Hg)	72.94	±9.03	71.84	±10.19
Diabetes, no. (%)	15	(15%)	18	(18%)
Current smoking, no. (%)	8	(8%)	8	(8%)
Waist circumference (inches)	36.80	±4.74	—	
Body mass index (kg/m^2)	28.78	±5.53	29.12	±6.18
ASCVD risk score	3.95	±4.87	8.77	±9.78
Medication use				
Prednisone, no. (%)	29	(29%)	14	(14%)
Methotrexate, no. (%)	78	(78%)	71	(71%)
No. concurrent csDMARDs	1.89	±0.78	1.70	± 0.99
bDMARD, no. (%)	63	(63%)	61	(61%)
Statin, no. (%)	39	(39%)	47	(47%)
Coronary plaque				
Plaque presence (any), no. (%)	69	(69%)	69	(69%)
Number of plaques total	1.89	±2.27	2.48	±3.02
Number of non-calcified plaques	0.96	±1.05	0.59	±0.81
Number of partially calcified plaques	0.55	±1.32	0.77	±1.61
Number of fully calcified plaques	0.38	±0.97	1.12	±1.86
Coronary artery calcium score	72.61	±284.68	144.30	±456.77

Values are mean±SD or N (per cent).
ACPA, anticitrullinated protein antibodies; ASCVD, atherosclerotic cardiovascular disease score; bDMARD, biologic disease-modifying antirheumatic drug; CLC, cholesterol loading capacity; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; DAS28-CRP, 28-joint disease activity score with C reactive protein; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; RF, rheumatoid factor.

−1.81±3.69 µg cholesterol/mg protein, p=0.0498). CLC change was not different in patients with versus without partially calcified plaque progression (−1.63±3.38 vs −1.50±3.80 µg cholesterol/mg protein, p=0.882) or total plaque progression (−1.00±3.52 vs −1.99±3.79 µg cholesterol/mg protein, p=0.178).

Higher CLC change is linked to accelerated plaque progression

CLC change (per SD unit increase) predicted non-calcified, calcified plaque, CAC progression and new extensive or obstructive disease (figures 1 and 2). Specifically, CLC change is associated with a greater likelihood

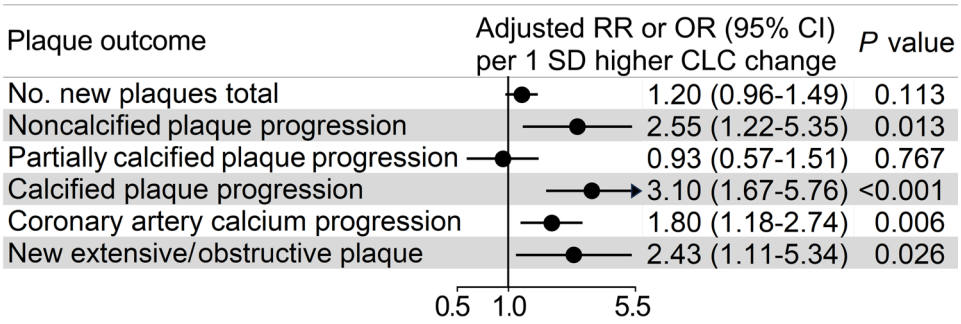

Figure 1 Impact of CLC change on plaque progression. CLC, cholesterol loading capacity; RR, rate ratio.

of new non-calcified (OR 2.55, 95% CI 1.22 to 5.35, p=0.013) and fully calcified plaque formation (OR 3.10, 95% CI 1.67 to 5.76, p≤0.001, figures 1 and 2A) after adjusting for ASCVD risk score, follow-up duration, baseline plaque, obesity and prednisone use during follow-up. Likewise, CLC change is associated with a greater likelihood of CAC progression (OR 1.80, 95% CI 1.18 to 2.74, p=0.006, figures 1 and 2B) after adjustments for ASCVD score, follow-up duration, baseline CAC, time-averaged CRP and obesity. CLC change is also associated with the likelihood of new extensive or obstructive disease after adjusting for ASCVD score, follow-up duration, baseline plaque and time-averaged CRP (OR 2.43, 95% CI 1.11 to 5.34, p=0.026, figures 1 and 2C). CLC change was not associated with a change in the number of total plaques or the progression of partially calcified plaque (figure 1).

Medication exposure influences the effect of CLC change on plaque progression

Greater CLC change was associated with 2.5 times increased likelihood of higher-risk partially calcified plaque progression in prednisone users and a 52% lower likelihood of such plaque progression in non-users (p-for-interaction=0.004, table 2) after adjusting for ASCVD score, follow-up duration and baseline plaque. In contrast, in prednisone non-users but not users, CLC change was associated with a four times greater likelihood of CAC progression (p-for-interaction=0.009) and a numerically greater likelihood of more stable, fully calcified plaque progression after adjusting for ASCVD score, follow-up duration, baseline plaque, time-averaged CRP and statin exposure throughout follow-up.

Greater CLC change is associated with a higher likelihood of non-calcified plaque progression in bDMARD exposed but not unexposed patients (p-for-interaction=0.003) and in statin exposed but not naive patients (p-for-interaction=0.048) after adjustments for ASCVD score, follow-up duration, baseline plaque, obesity and prednisone exposure throughout follow-up (table 2). Moreover, CLC change is associated with a greater likelihood of CAC progression in statin users but not in non-users (p-for-interaction=0.040) after adjusting for ASCVD score, follow-up duration, baseline CAC, time-averaged CRP, prednisone exposure and obesity. In contrast, CLC change predicted a greater likelihood of new extensive or obstructive plaque in statin non-users but not users (p-for-interaction=0.048) after adjusting for ASCVD score, follow-up duration, baseline plaque and time-averaged CRP.

DISCUSSION

This study is the first to examine the influence of changes in macrophage CLC on coronary atherosclerosis progression in RA or any disease state or population. This addresses an important clinical knowledge gap, as CLC has been linked to atherosclerosis burden and cardiovascular risk in RA, independently of LDL levels.[10] Previous studies have shown that plaque progression, which is more pronounced in RA,[2] predicts cardiovascular event

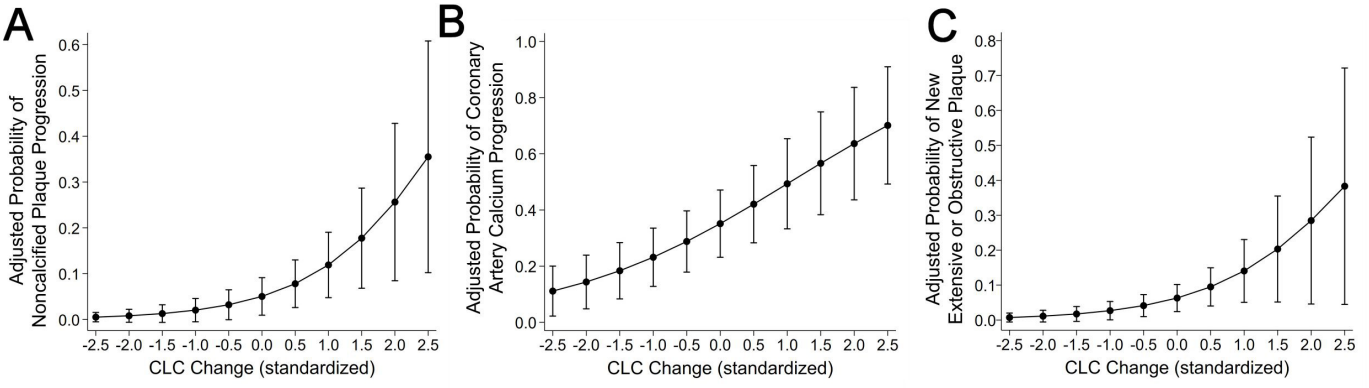

Figure 2 Association of CLC change with adjusted probability of (A) non-calcified plaque progression, (B) coronary artery calcium progression and (C) new extensive or obstructive plaque. CLC, cholesterol loading capacity.

Table 2 Exposure to prednisone, bDMARDs and statins during follow-up influences the effect of CLC change on plaque progression

	RR or OR (95% CI; p-value) per SD higher CLC change		
	Medication unexposed	Medication exposed	P-Int.
Number of new plaques total			
Prednisone	0.99 (0.67 to 1.47; p=0.978)	1.44 (0.97 to 2.14; p=0.070)	0.211
bDMARDs	0.95 (0.62 to 1.46; p=0.821)	1.28 (0.94 to 1.74; p=0.122)	0.316
Statins	1.32 (0.94 to 1.86; p=0.104)	1.16 (0.88 to 1.53; p=0.285)	0.553
Non-calcified plaque progression			
Prednisone	2.85 (0.79 to 10.30; p=0.109)	1.45 (0.67 to 3.10; p=0.345)	0.375
bDMARDs	0.81 (0.27 to 2.39; p=0.701)	4.27 (1.80 to 10.17; p=0.001)	0.003
Statins	1.27 (0.46 to 3.50; p=0.638)	5.41 (1.84 to 15.84; p=0.002)	0.048
Partially calcified plaque progression			
Prednisone	0.48 (0.23 to 0.99; p=0.048)	2.50 (1.19 to 5.27; p=0.016)	0.004
bDMARDs	0.88 (0.42 to 1.83; p=0.733)	0.94 (0.52 to 1.68; p=0.834)	0.892
Statins	1.30 (0.60 to 2.79; p=0.509)	0.79 (0.43 to 1.47; p=0.465)	0.329
Calcified plaque progression			
Prednisone	5.58 (2.09 to 14.88; p=0.001)	2.25 (0.91 to 5.60; p=0.081)	0.158
bDMARDs	2.78 (0.77 to 10.03; p=0.118)	3.79 (1.98 to 7.26; p<0.001)	0.660
Statins	3.93 (1.64 to 9.47; p=0.002)	3.05 (1.38 to 6.71; p=0.006)	0.662
Coronary artery calcium progression			
Prednisone	4.13 (1.86 to 9.15; p<0.001)	0.98 (0.50 to 1.92; p=0.962)	0.009
bDMARDs	2.10 (0.88 to 4.97; p=0.093)	1.98 (1.10 to 3.59; p=0.023)	0.761
Statins	1.22 (0.68 to 2.19; p=0.513)	3.86 (1.56 to 9.55; p=0.004)	0.040
New extensive/obstructive plaque			
Prednisone	2.74 (0.60 to 12.52; p=0.193)	1.37 (0.73 to 2.58; p=0.324)	0.416
bDMARDs	1.36 (0.30 to 6.23; p=0.688)	2.04 (0.99 to 4.21; p=0.054)	0.644
Statins	4.11 (2.07 to 8.19; p=<0.001)	1.48 (0.66 to 3.31; p=0.335)	0.048

bDMARDs, biologic disease-modifying antirheumatic drugs; CLC, cholesterol loading capacity; P-int., P value for CLC change by medication exposure interaction; RR, rate ratio.;

risk independently of baseline plaque.[11 12 25] We observed that patients with atherosclerosis progression had greater CLC change compared with those without. CLC change was linked to non-calcified and calcified plaque progression, increased CAC and new extensive or obstructive plaque, the latter indicating very high cardiovascular risk in both patients with RA and controls.[19 20 26] These findings suggest that novel therapies targeting cholesterol loading and handling within macrophage foam cells in established atherosclerotic lesions could complement conventional preventive therapies to reduce cardiovascular risk.[8 27 28] Notably, bDMARDs used to treat RA, such as TNFi and tocilizumab, may reduce cholesterol loading on macrophages independently of their anti-inflammatory effects.[22 29] For example, adalimumab has been shown to decrease cholesterol uptake in lipopolysaccharide-primed human macrophages by binding to surface TNF, leading to internalisation and reverse signalling.[22]

CLC decreased in 68% of patients in our study. Improvements in systemic inflammation, oxidation and immunomodulator use may positively affect lipoprotein structure and function, leading to reductions in CLC and a shift towards normal cholesterol uptake through the LDL receptor.[8 22 29 30] Indeed, greater reductions in CLC were associated with lower cumulative inflammation measured by time-averaged ESR. This aligns with our earlier report that increased inflammation augments the effect of oxLDL on CLC.[8] We previously found that cumulative inflammation independently predicts coronary plaque progression in RA.[2] Here, we extend those findings by showing that reductions in CLC, reflecting lower cumulative inflammation, are linked to decreased atherosclerosis progression. The protective effect of CLC reduction is further supported by improvement in high-density lipoprotein (HDL) function. Specifically, in supplementary analyses, we showed that CLC change was inversely associated with a change in ATP-binding

cassette transporter G1 (ABCG1)-mediated cholesterol efflux (results not shown). Consequently, greater reductions in macrophage cholesterol loading over time coincided with greater increases in efflux of cholesterol out of arterial wall macrophages. This is consistent with our earlier finding of an inverse relationship between CLC and ABCG1-mediated cholesterol efflux.[31]

Patients with higher baseline CLC demonstrated greater reductions at follow-up. This could be explained in part by higher baseline inflammation in these patients, which might render them more responsive to antirheumatic therapies that improve lipoprotein function. Indeed, we previously reported that inflammation promoted macrophage cholesterol loading via oxLDL through upregulation of anti-oxLDL IgG and proprotein convertase subtilisin kexin type-9.[8] Moreover, tocilizumab was shown to induce the greatest CLC reductions in patients with the highest baseline levels.[29] Another possible explanation for this finding may reflect tissue-level changes. The number of foam cells in atherosclerotic lesions increases with plaque growth and decreases with regression,[32–34] while foam cell size and granularity increase in response to lipid droplet accumulation.[35] Consistent with this, we found that higher baseline CLC was associated with greater atherosclerosis burden at baseline.[10] Yet, upon excess lipid loading, foam cells undergo extensive transcriptional reprogramming, downregulating proinflammatory genes and increasing expression of genes involved in cholesterol processing and efflux.[35–37] This reprogramming leads to the development of a profibrotic phenotype, which has been associated with plaque stability in animal models.[38] Thus, the highest baseline CLC, while reflecting past systemic inflammation and oxidation, may also coincide with the onset of this beneficial transcriptional reprogramming in foam cells. As immunomodulatory treatments reduce the recruitment of new inflammatory cells to plaques and improve the antiatherogenic function of circulating LDL and HDL,[39 40] plaque progression may slow and even regress.[41] This reduction in proinflammatory activity of macrophages by immunomodulatory therapies, as indicated by decreasing CLC, may further improve systemic inflammation and lipoprotein function. The association between greater reductions in CLC and lower cumulative inflammation supports this hypothesis.

Although we did not observe differences in CLC change between patients exposed or unexposed to prednisone, bDMARDs or statins, these therapies influenced the relationship between CLC change and atherosclerosis progression. Previous reports demonstrated that prednisone use independently predicts coronary atherosclerosis progression[2] and cardiovascular risk in RA.[16] Greater CLC change was linked to increased high risk, partially calcified plaque progression in prednisone users, while in non-users, it is associated with decreased partially calcified plaque progression and greater progression of CAC and stable fully calcified plaque. Prednisone contributes to excessive intracellular cholesterol via macrophage loading through the LDL receptor[42] and increases expression of macrophage migration inhibitory factor, which may offset some of its anti-inflammatory effects.[43] In fact, migration inhibitory factor has been shown to promote monocyte influx, macrophage activation and cytokine production in plaques and lead to more severe RA and atherosclerosis.[43 44] Consequently, increasing CLC in the context of a greater arterial macrophage burden facilitated by corticosteroid use could accelerate plaque progression.

We also observed that greater CLC change is associated with a higher likelihood of lipid-rich non-calcified plaque formation, especially in bDMARD and statin users. Since previous studies have reported that, in principle, bDMARDs and statins reduce CLC[22 29 45 46] and decrease non-calcified plaque progression,[14 15] our current results suggest that increasing CLC notwithstanding treatment with these agents may identify extremely high-risk subgroups. It is conceivable that a greater inflammatory burden[2 47] or presence of extra-articular disease[48] may underlie such increases in CLC over time and despite treatment with bDMARDs and statins. However, in sensitivity analyses, CLC change was not associated with the presence of rheumatoid nodules or interstitial lung disease or an expanded definition of extra-articular disease including rheumatoid nodules, interstitial lung disease, Sjogren's syndrome, scleritis, peripheral neuropathy and vasculitis. Moreover, we found no differences in either baseline or time-averaged DAS28CRP, CRP or ESR in bDMARD users with increasing versus decreasing CLC (data not shown). Indeed, in these very high-risk patients, increasing CLC could still result in greater non-calcified plaque formation. However, this increase was accompanied by accelerated calcification and stabilisation, evidenced by greater calcified plaque and CAC progression. This is consistent with our prior work showing accelerated calcification of both prevalent and incident plaques in patients treated with bDMARDs and statins.[14 15] Notably, greater CLC change predicted new extensive or obstructive disease only in statin non-users, in line with prior reports of statins attenuating non-calcified plaque progression or even fostering regression[15] and further supporting their protective role in preventing high-risk plaque.

Our study has certain limitations. The effect of CLC change on plaque progression was not a prespecified analysis in our original study design,[1] so these findings should be considered exploratory. They should therefore be validated in adequately powered future studies, preferably including a non-autoimmune disease control group, to extend the presence and relevance of these findings in other populations. Most patients in our cohort had established RA and self-identified as white Hispanic, limiting the generalisability of our findings to other populations. As CLC assessments were performed on both baseline and follow-up samples simultaneously, the baseline samples had been in storage longer, and this may have affected measurement accuracy. However, the available literature suggests that significant lipoprotein

modification is unlikely in storage at −70°.[49 50] Finally, while CLC reductions might reflect regression to the mean, the consistent directional associations observed between CLC change and plaque progression support the robustness of our findings.

CONCLUSION

Increases in CLC, reflecting enhanced serum lipoprotein capacity to load cholesterol in arterial macrophages, are associated with coronary atherosclerosis progression in a dose-dependent manner, including new high-risk non-calcified and extensive or obstructive plaque. Therapies designed to decrease or inhibit cholesterol loading and uptake by macrophages within established atherosclerotic lesions may complement existing preventive and anti-inflammatory strategies, offering a more comprehensive approach to cardiovascular risk reduction in RA.

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