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Inflammation and immunomodulatory therapies influence the relationship between ATP-binding cassette A1 membrane transporter-mediated cholesterol efflux capacity and coronary atherosclerosis in rheumatoid arthritis

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ABSTRACT

Objectives: High-density lipoprotein (HDL) removes cholesterol from cells in atherosclerotic lesions, a function known as cholesterol efflux capacity (CEC). ATP-binding-cassette A1 (ABCA1) membrane transporter starts cholesterol transfer from macrophages to HDL particles. In rheumatoid arthritis (RA), methotrexate and biologic disease modifying drugs (bDMARDs) are atheroprotective whereas corticosteroids and C-reactive protein (CRP) are proatherogenic. We evaluated the influence of these factors on the relationship of ABCA1-CEC with atherosclerosis and cardiovascular events.

Methods: Atherosclerosis was evaluated with computed tomography angiography in 140 patients with RA and repeated in 99 after 6.9 ± 0.3 years. Events including acute coronary syndromes, stroke, cardiovascular death, claudication, revascularization, and heart failure were recorded. ABCA1-CEC was quantified in J774A.1 murine macrophages and reported as percentage of effluxed over intracellular cholesterol.

Results: Higher ABCA1-CEC associated with (i) more calcified plaques at baseline only in patients with CRP>7 mg/L (median) (p-interaction = 0.001) and methotrexate nonusers (p-interaction = 0.037), and more partially-calcified plaques only in bDMARD nonusers (p-interaction = 0.029); (ii) fewer new calcified plaques in patients with below-median but not higher time-averaged CRP (p-interaction = 0.028); (iii) fewer new total and calcified plaques in prednisone unexposed but not patients exposed to prednisone during follow-up (p-interaction = 0.034 and 0.004) and (iv) more new plaques in baseline bDMARD nonusers and fewer in bDMARD users (p-interaction ≤ 0.001). Also, ABCA1-CEC associated with greater cardiovascular risk only in baseline prednisone users (p-interaction = 0.027).

Conclusion: ABCA1-CEC associated with decreased atherosclerosis in patients with below-median baseline and time-averaged CRP and bDMARD use. Conversely, ABCA1-CEC associated with increased plaque in those with higher CRP, corticosteroid users, methotrexate nonusers, and bDMARD nonusers. While in well-treated and controlled disease ABCA1-CEC appears atheroprotective, in uncontrolled RA its action may be masked or fail to counteract the inflammation-driven proatherogenic state.

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1. Introduction

Cholesterol concentration in arterial wall macrophages is determined by its loading through native or modified low-density lipoprotein (LDL) and its outflow, in a process referred to as reverse cholesterol transport [1]. Cholesterol efflux is the initial step in this process and reflects the capacity of high-density lipoprotein (HDL) to remove cholesterol from foam cells (cholesterol efflux capacity, CEC) [1]. Members of the ATP-binding-cassette membrane (ABC) transporter family expressed on the surface of macrophages actively export cholesterol to diverse HDL particles [1]. Specifically, ABCA1 initiates cholesterol export to apo-A1 or lipid-poor discoidal pre-β HDL particles; with the help of serum lecithin cholesterol acyl transferase (LCAT), these particles mature into spherical HDL which subsequently accept additional cholesterol via the ABCG1 membrane transporter. Overall, CEC inversely associated with atherosclerotic lesion size, lipid content and macrophage burden, and more importantly cardiovascular morbidity and mortality in general patients, independently of HDL-C levels [2–4]. However, studies reporting on CEC differ in their use of experimental models, cholesterol transporters involved, and type of HDL particles evaluated. These should be considered when comparing CEC in patients and controls or when studying the effects of disease activity or medications on this HDL function. Consequently, available data have not yielded definitive conclusions in rheumatoid arthritis (RA) [5]. Nevertheless, it was shown that CEC was negatively linked to carotid plaque presence and recovered with therapy in this disease [6]. Yet, the contributions of individual CEC pathways to coronary atherosclerosis and cardiovascular risk in RA have not been adequately characterized.

We reported that ABCG1-CEC was reduced in RA, inversely associated with disease activity [7], and improved with therapy [5,8]. It negatively associated with coronary atherosclerosis burden, plaque progression and cardiovascular risk provisionally on lower cumulative inflammation and corticosteroid dose [9]. In contrast, ABCA1-CEC directly associated with cardiovascular risk and statin use influenced the effect of ABCA1-CEC on atherosclerosis as well as event risk [10]. In statin nonusers, higher ABCA1-CEC associated with greater atherosclerosis burden, whereas in users ABCA1-CEC inversely associated with plaque burden and progression [10].

Inflammation and corticosteroid use are proatherogenic [11,12] while methotrexate and biologic DMARDs (bDMARDs) are atheroprotective [13,14]. However, whether and to what extent do disease-related inflammation and immunomodulatory therapies influence ABCA1-CEC or its relationship with coronary atherosclerosis is unknown. In this study we explored whether inflammation, corticosteroids, methotrexate and bDMARDs impact on ABCA1-CEC or its relationship with coronary atherosclerosis burden, progression and long-term cardiovascular risk.

2. Material and methods

2.1. Patient recruitment

We included 140 patients formerly registered in the *PRO*spec*T*ive *E*valuation of Latent *C*oronary A*T*herosclerosis in *R*heumatoid *A*rthritis (PROTECT RA) cohort [15] who had serum available for CEC evaluation. The original cohort encompassed 150 patients with regular follow-up in a single academic hospital, prospectively undergoing noninvasive coronary atherosclerosis assessment with computed tomography angiography (CCTA). Subjects were then followed for incident cardiovascular events; 99 underwent serial evaluation for plaque progression 6.9 ± 0.3 years later. Patients were enrolled if they were between 18 and 75 years old, satisfied 2010 classification criteria for RA and had no prior history of cardiovascular disease including angina, acute coronary syndromes, transient ischemic attack, stroke, claudication, revascularization, or heart failure. Patients with concomitant autoimmune diseases (except for Sjogren's), active or chronic infections, malignancy within 5 years, body weight greater than 147.7 kg (scanner capacity), glomerular filtration rate <60 mL/min, or allergy to iodinated compounds were excluded. The study was approved by the local Institutional Review Board and all subjects signed informed consent in accordance with the Declaration of Helsinki.

2.2. Coronary computed tomography angiography

Screening coronary atherosclerosis assessments were carried out in a 64-multidetector row scanner between March 2010 and March 2011. Serial evaluations were completed in a 256-multidetector row scanner from March 2017 to March 2018. Standard operating procedures regarding image acquisition, processing and grading reproducibility have been previously outlined [14]. Coronary artery calcium score was measured according to Agatston [16]. Atherosclerosis presence was evaluated on contrast-enhanced scans according to a standardized 17-segment American Heart Association model [17]. Both screening and serial studies were reviewed at the same time and in random order by an experienced and blinded reader (MJB). Longitudinal comparisons were carried out with coronary segment coalignment using fixed anatomic landmarks as fiducial points. Segment involvement score depicted the total number of coronary segments harboring plaque in an individual patient (0-17). Plaque stenosis severity in each segment was rated on a scale from 0 to 4 as previously reported and segment stenosis score described the cumulative stenotic severity of all evaluable segments in an individual patient (0-68) [15]. Plaque composition was reported as noncalcified, partially and fully-calcified as previously described [15].

2.3. Laboratory evaluations

Laboratory assessments including complete blood counts, a metabolic panel, erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP) were completed at the time of screening and serial coronary assessments as well as every clinic visit during follow-up. Fasting lipid measurements were similarly performed at the time of baseline and follow-up scans and based on EULAR recommendations in between [18]. Additional serum for biomarker studies was stored as previously described [15].

2.3.1. Serum cholesterol efflux capacity

ABCA1-CEC was evaluated in J774A.1 murine macrophages cultured in the absence or presence of c-AMP (Merck Life Science, Darmstadt, Germany) as previously reported [19]. Briefly, cells were first labeled with 1,2–^{3}H(N)-cholesterol for 24 h and then incubated in medium with 0.2% bovine serum albumin for an additional 18 h. Apo-B depleted serum 2% (vol/vol) was added next for 4 h [20]. CEC was expressed as the proportion of radioactivity present in the supernatant over the total intracellular radioactivity. The difference in CEC between cells cultured in the presence or absence of c-AMP represented the ABCA1-specific CEC contribution. A pool of human normolipidemic sera and 10 μg/ml of human isolated apo-AI (Merk Life Science, Darmstadt, Germany) were also tested in each assay to control for inter-assay variability and normalize sample values across various experiments. The normalized CEC from human normolipidemic sera and human apoA-I also provided an index of intra-assay variability (calculated as <10%).

2.4. Covariates and outcomes

All patients had a 10-year atherosclerotic cardiovascular disease (ASCVD) risk score calculated at baseline based on the American Heart Association pooled cohort equation [21]. Height, weight and waist circumference were collected. Disease activity was calculated based on tender and swollen joint counts on a 28-joint assessment and C-reactive protein (DAS28-CRP) at all clinic visits (every three to four months). Methotrexate, bDMARD, prednisone and statin use and dosing was recorded on each visit and confirmed against pharmacy records. Weighted average prednisone dose was calculated by dividing the

cumulative dose exposure for each patient with the total number of days of follow-up. 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 [22].

Coronary plaque outcomes of interest comprised numbers of segments with any, noncalcified, partially and fully calcified plaques at baseline and follow-up. The composite endpoint of cardiovascular death, acute coronary syndrome, stroke, peripheral vascular disease, revascularization, and new onset heart failure was the prespecified clinical outcome.

2.5. Statistical analysis

Categorical variables were presented as frequencies with percentages and continuous ones as means with standard deviations (SD). Non-normally distributed variables were natural log transformed. The effect of ABCA1-CEC on the number of atherosclerotic lesions at baseline and their change at follow-up was evaluated with robust negative binomial regression. The influence of baseline CRP, time-averaged CRP, baseline prednisone use, weighted daily average prednisone dose, baseline methotrexate use, and baseline bDMARD use on the association between ABCA1-CEC and plaque outcomes was interrogated by adding the corresponding moderators and their products with ABCA1-CEC as interaction terms in the respective models. Baseline plaque outcome models adjusted for ASCVD score and statin use; individual models further adjusted for covariates significant in the corresponding multivariable models. Plaque progression models covaried for ASCVD score, number of the respective plaque types at baseline, as well as covariates significant in the corresponding multivariable models.

Cox regression models evaluated baseline CRP, prednisone use, methotrexate use, and bDMARD use as potential moderators of the relationship between ABCA1-CEC and cardiovascular event risk. Each model included ASCVD, extensive or obstructive plaque at baseline, ABCA1-CEC, the respective moderator, and the interaction term of the moderator with ABCA1-CEC. SPSS version 27 and Stata version 15 were used. P values < 0.05 were considered significant.

3. Results

Patients were in general middle-aged females with established, seropositive and erosive disease. They received on average two concurrent csDMARDs (80% methotrexate) and 86/140 (61%) were additionally treated with bDMARDs (all TNF-α inhibitors). Patient characteristics appear in Table 1. Mean (standard deviation [SD]) ABCA1-CEC was 5.14 (1.07)%. Ninety-eight patients (70%) had atherosclerosis on screening CCTA. ABCA1-CEC was not differemt between patients with and without atherosclerosis [5.10 (4.88–5.32)% vs. 5.22 (4.91–5.53)%, p = 0.53] or in those with vs. without specific plaque types even after adjustments for ASCVD score and DAS28-CRP (not shown).

Table 1
Baseline sample characteristics (N = 140).

	Mean ± SD or N (%)	
Age (years)	52.91	±10.52
Female, no. (%)	123	(87.86)
RA duration (years)	10.58	±7.61
Age at diagnosis (years)	42.33	±11.15
RF positive, no. (%)	121	(86.43)
ACPA positive, no. (%)	120	(85.71)
Erosions, no. (%)	92	(65.71)
ESR (mm/hr)	27.11	±17.85
CRP (ln) (mg/dL)	1.49	±1.15
DAS28-CRP	169.25	±35.25
Total cholesterol (mg/dL)	2.53	±0.99
LDL-c (mg/dL)	95.04	±28.37
HDL-c (mg/dL)	51.49	±14.08
Triglycerides (mg/dL)	146.53	±89.23
Systolic BP	128.35	±15.34
Diastolic BP	73.17	±8.91
Diabetes, no. (%)	22	(15.71)
Current smoking, no. (%)	12	(8.57)
Body mass index (kg/m^2)	28.95	±5.60
Waist circumference (inches)	36.73	±4.84
ASCVD risk score	4.96	±6.83
Prednisone use, no. (%)	48	(34.29)
Methotrexate use, no. (%)	112	(80.00)
No. concurrent csDMARDs	1.94	±0.80
bDMARD use, no. (%)	86	(61.43)
Plaque presence (any), no. (%)	98	(70.00)
Number of plaques total	1.96	±2.27
Number of noncalcified plaque	1.01	±1.16
Number of partially calcified plaques	0.56	±1.30
Number of fully calcified plaques	0.42	±1.05
ABCA1-CEC (%)	5.14	±1.07

Values are mean ± standard deviation (SD) unless otherwise indicated. RA: rheumatoid arthritis, RF: rheumatoid factor, ACPA: anti-citrullinated protein antibodies, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, DAS28-CRP: disease activity score based on 28 joint counts and CRP, LDL-c: low-density lipoprotein cholesterol, HDL-c: high density lipoprotein cholesterol, BP: blood pressure, ASCVD: atherosclerotic cardiovascular disease score, cs-DMARDs: conventional synthetic disease modifying anti-rheumatic drugs, bDMARD: biologic disease modifying anti-rheumatic drugs, ABCA1-CEC: Cholesterol efflux capacity through the ABCA1 transporter.

3.1. Moderators of the association of ABCA1-CEC with baseline atherosclerosis

ABCA1-CEC inversely associated with baseline CRP (r = −0.182, p = 0.032) and DAS28-CRP (r = −0.171, p = 0.044) even after adjustments for ASCVD score and statin use. Baseline CRP influenced the relationship between ABCA1-CEC and fully-calcified plaque at screening (p-for-interaction = 0.001, Fig. 1A); higher ABCA1-CEC (per 1-SD increment) associated with more fully calcified plaques in patients with CRP>7 mg/L (median) (incidence rate ratio [IRR] 2.24, [95% CI 1.41–3.56], p = 0.001) and fewer fully calcified plaques in those with CRP<7 mg/L (IRR 0.52 [95% CI 0.32–0.83], p = 0.007) after adjusting for ASCVD score, waist circumference, statin use, and prednisone use. There were no significant interactions between ABCA1-CEC and CRP predicting other plaque types (not shown). Additionally, higher ABCA1-CEC associated with more fully calcified plaques in baseline methotrexate nonusers (IRR 2.25 [95% CI 1.08–4.64], p = 0.029) but not in users (IRR 0.88 [95% CI 0.58–1.33], p = 0.532) after adjusting for ASCVD, CRP, statin use, prednisone use, and waist circumference (p-for-interaction = 0.037, Fig. 1B). Similarly, after adjusting for ASCVD, statin use and age at RA diagnosis, higher ABCA1-CEC associated with more partially calcified plaques in baseline bDMARD nonusers (IRR 2.43 [95% CI 1.30–4.53], p = 0.005, Fig. 1C) but not users (IRR 1.12 [95% CI 0.82–1.53], p = 0.490; p-for-interaction = 0.029). There were no significant interactions between ABCA1-CEC and baseline methotrexate, bDMARD, or prednisone use predicting other plaque outcomes (not shown).

3.2. Moderators of the association of ABCA1-CEC with atherosclerosis progression

Follow-up assessments for atherosclerosis progression were carried out in 99 of 140 patients within 6.9 ± 0.3 years. Of the 41 patients without reevaluation, two expired, six migrated, four were lost to follow-up after the screening assessment, and 29 declined. Although patients lacking surveillance assessments were older, with higher systolic blood pressure and ASCVD, the differences in ASCVD scores were no longer significant after adjusting for age (Supplementary Table S1). Coronary atherosclerosis was present in 68/99 (68.7%) patients at follow-up. Of those, 10 (14.7%) had no atherosclerosis at baseline and formed a total of 15 new plaques at follow-up. The remaining 58 patients with atherosclerosis at baseline displayed an additional 84 new plaques

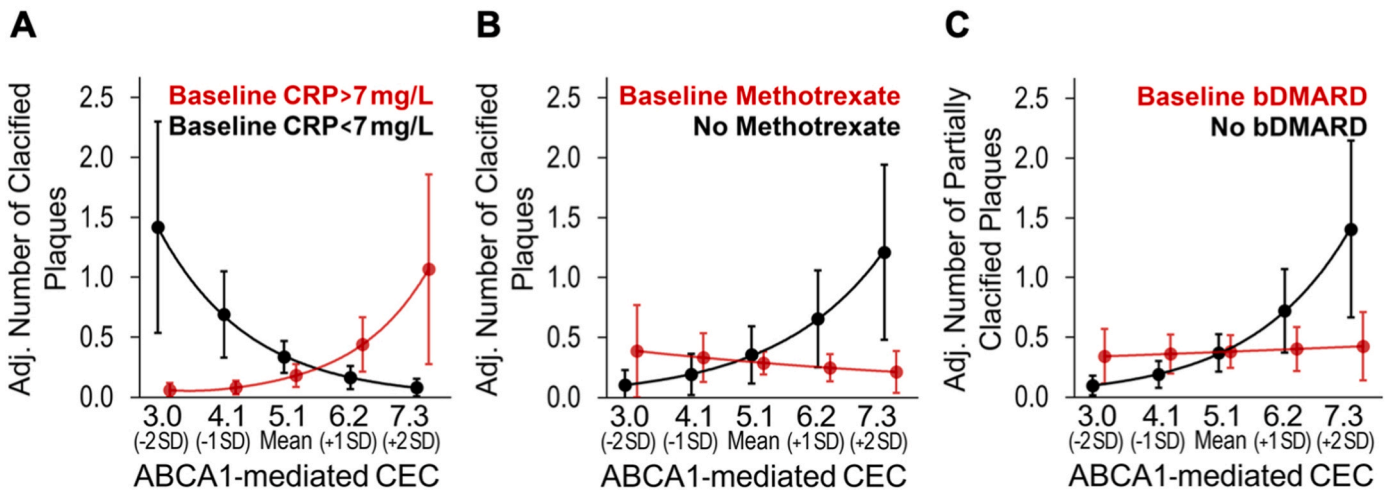

Fig. 1. Impact of inflammation (A) and disease-specific therapies such as Methotrexate (B) and bDMARDs (C) on the relationship between ABCA1-CEC and baseline plaque burden in RA.
ABCA1-CEC, cholesterol efflux capacity through the ATP-binding cassette A1 pathway.

at follow-up.

3.2.1. Influence of CRP on the effect of ABCA1-CEC on plaque progression

Higher ABCA1-CEC associated with fewer new fully calcified plaques only in patients with time-averaged CRP<7 mg/L (median) after adjustments for ASCVD, baseline plaque, statin duration, and waist-to-height ratio (p-for-interaction = 0.028, Fig. 2). Indeed, patients with time-averaged CRP<7 mg/L had fewer new fully calcified plaques compared to those with time-averaged CRP>7 mg/L as ABCA1-CEC increased (Fig. 3A). There were no significant interactions of ABCA1-CEC with time-averaged CRP predicting other plaque progression outcomes (not shown).

3.2.2. Influence of prednisone use on the effect of ABCA1-CEC on plaque progression

DAS28-CRP and CRP were higher in baseline prednisone users than nonusers (2.95 ± 1.17 vs. 2.31± 0.81, p < 0.001 and 11.92± 13.66 vs. 6.97± 11.68 mg/L, p = 0.026 respectively). Likewise, patients exposed to prednisone during follow-up had higher time-averaged DAS28-CRP and time-averaged CRP compared to nonusers (3.16± 0.83 vs. 2.24± 0.58, p < 0.001 and 9.12± 2.20 vs. 6.17± 1.90 mg/L, p = 0.008 respectively) and time-averaged CRP associated with the time-weighted average prednisone dose (b = 0.08 [95% CI 0.02–1.43], p = 0.013) after adjustments for ASCVD score, numbers of plaques at baseline, statin duration, and baseline CRP.

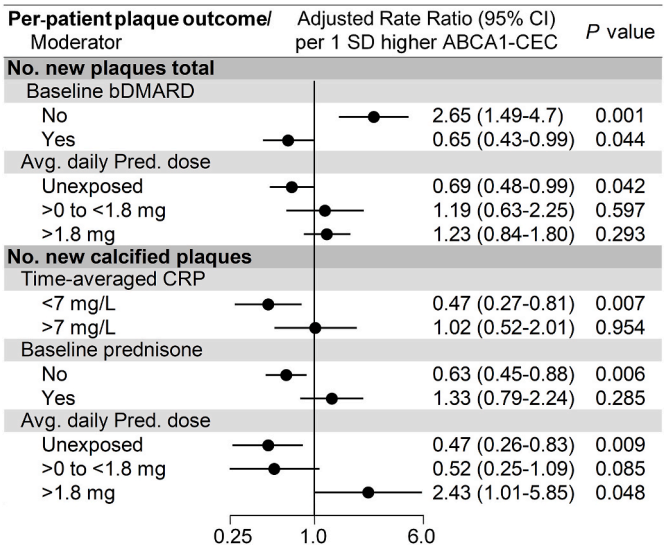

Fig. 2. Impact of inflammation and disease-specific therapies on the relationship between ABCA1-CEC and plaque progression in RA.
ABCA1-CEC, cholesterol efflux capacity through the ATP-binding cassette A1 pathway; SD, standard deviation.

Higher ABCA1-CEC associated with fewer new fully calcified plaques in baseline prednisone nonusers but not in users after adjustment for ASCVD score, baseline plaque, time-averaged CRP, and waist-to-height ratio (p-for-interaction = 0.021, Fig. 2 and 3B). There were also significant interactions of ABCA1-CEC with prednisone exposure during follow-up predicting plaque progression. Specifically, ABCA1-CEC associated with fewer new plaques only in prednisone unexposed patients after controlling for ASCVD score, baseline plaque, statin duration, and time-averaged CRP (p-for-interaction = 0.034, Fig. 2). In contrast, as ABCA1-CEC increased, those receiving the highest time-weighted average prednisone dose (>1.8 mg/d) had more new plaques compared to unexposed patients (Fig. 3C). Additionally, higher ABCA1-CEC was linked to fewer new calcified plaques in prednisone unexposed patients and more new calcified plaques in those with time-weighted average prednisone dose >1.8 mg/d after controlling for ASCVD score, baseline plaque, statin duration, time-averaged CRP, and waist-to-height ratio (p-for-interaction = 0.004, Fig. 2 and 3D). There were no significant interactions of ABCA1-CEC with baseline prednisone or prednisone exposure during follow-up predicting number of new noncalcified or partially calcified plaques (not shown).

3.2.3. Influence of baseline methotrexate and bDMARD use on the effect of ABCA1-CEC on plaque progression

Baseline methotrexate use did not moderate the effect of ABCA1-CEC on plaque progression. In contrast, ABCA1-CEC associated with more new plaques in baseline bDMARD nonusers and fewer new plaques in users after adjustments for ASCVD score, baseline plaque and time-averaged CRP (p for interaction ≤ 0.001, Fig. 2 and 3E). There were no significant interactions of ABCA1-CEC with baseline bDMARD use predicting other plaque progression outcomes (not shown).

3.3. Influence of CRP, prednisone, methotrexate, and bDMARD use on the effect of ABCA1-CEC on cardiovascular risk

Eighteen cardiovascular events were recorded in 15 patients over 6.03 ± 2.42 years of follow-up (incidence rate of 2.08 [1.31–3.30]

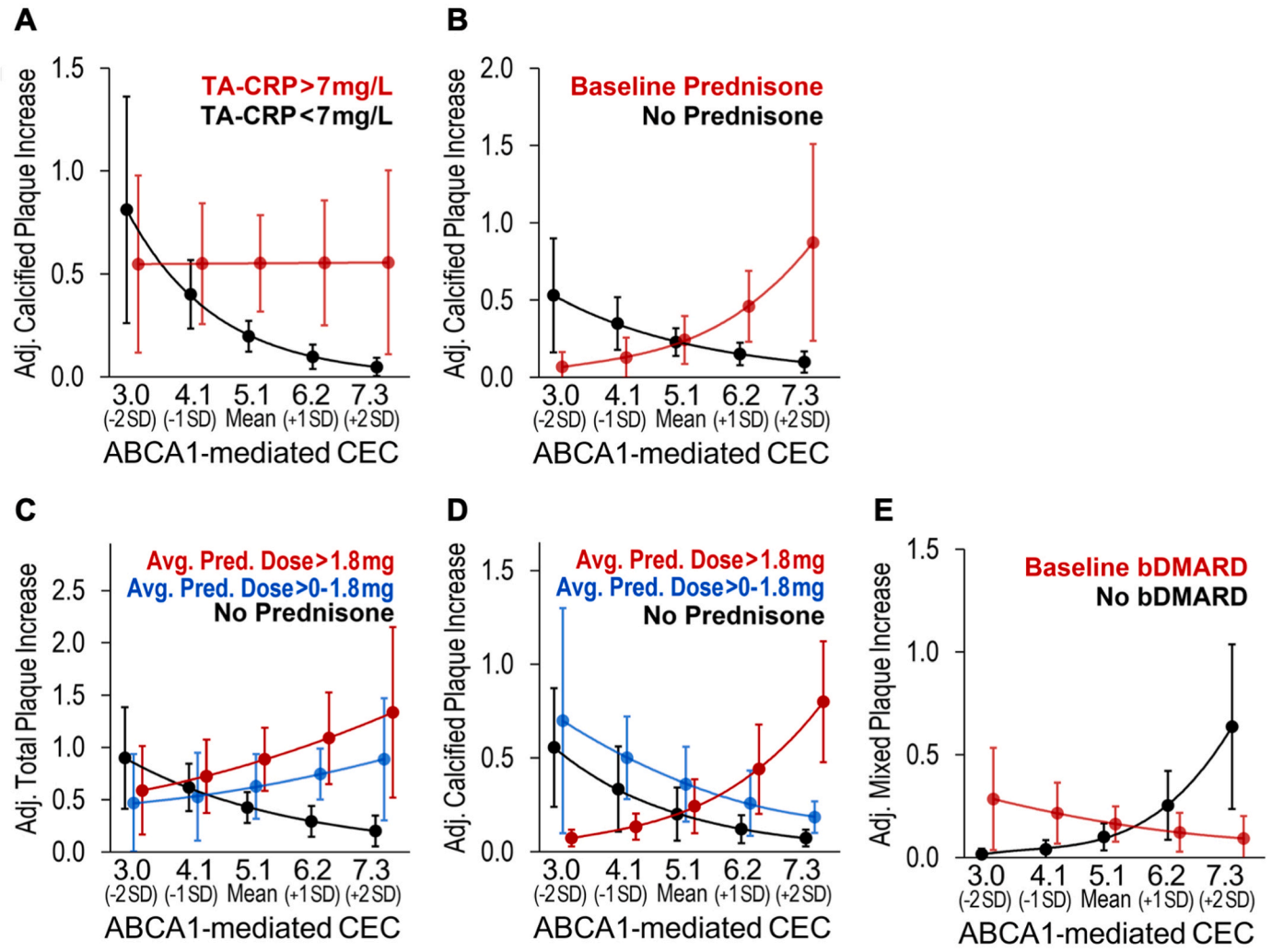

Fig. 3. Impact of time-averaged CRP (A) and disease-specific therapies such as baseline prednisone (B), daily-weighted average prednisone dose during follow-up on total (C) and fully-calcified plaque (D) and baseline bDMARD use (E) on the relationship between ABCA1-CEC and plaque progression in RA.
ABCA1-CEC, cholesterol efflux capacity through the ATP-binding cassette A1 pathway; SD, standard deviation.

events/100 patient-years, Supplementary Table S2). Baseline CRP did not influence the relationship between ABCA1-CEC and cardiovascular risk (p-for-interaction = 0.396). There was an interaction between ABCA1-CEC and baseline prednisone use (p-for-interaction = 0.027, Fig. 4); after adjusting for ASCVD score and baseline segment involvement score, higher ABCA1-CEC (per 1-SD increment) associated with greater cardiovascular risk in prednisone users (hazard ratio 5.56 [95% CI 2.11–14.70], p = 0.001) but not in nonusers (hazard ratio 1.68 [95% CI 0.97–2.93], p = 0.063). Neither baseline methotrexate nor bDMARD use influenced the effect of ABCA1-CEC on cardiovascular risk (p-for-interaction = 0.889 and 0.910 respectively).

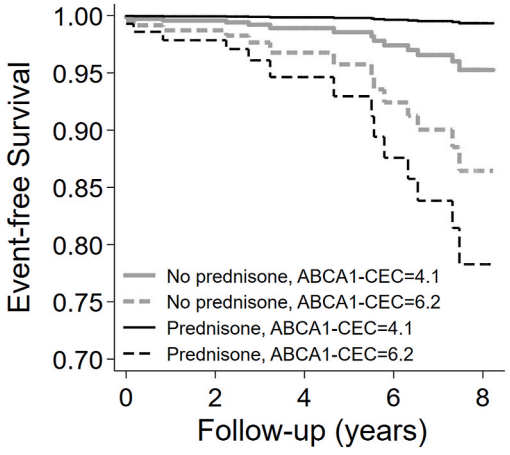

Fig. 4. Impact of high or low ABCA1-CEC on cardiovascular risk in baseline prednisone users and nonusers.
ABCA1-CEC, cholesterol efflux capacity through the ATP-binding cassette A1 pathway.

4. Discussion

The present study is the first to directly investigate the impact of inflammation and immunomodulatory therapies on ABCA1-CEC and its relationship with coronary atherosclerosis and cardiovascular risk. Findings highlight their contextual contributions and conditional regulation of these relationships and suggest mechanistic insights.

Increased ABCA1-CEC may result from greater pre-β HDL availability [23]. The latter may reflect either increased hepatic synthesis of ApoA1, recycling of pre-β HDL particles, or alternatively a block in pre-β HDL maturation to larger spherical HDL3 particles as seen in LCAT deficiency [24,25]. Indeed, LCAT deficiency was linked to high pre-β HDL and ABCA1-CEC, and low HDL_3 and ABCG1-CEC [24]. Decreased levels and activity of LCAT have been reported in RA patients, especially during

periods of high disease activity [26], and those improved with treatment [27]. Importantly, LCAT deficiency was linked to premature atherosclerosis and greater plaque burden [28,29]. The inverse association between ABCA1-CEC and ABCG1-CEC observed in our patient sample suggests a block in pre-β HDL maturation with disruption in effective cholesterol efflux and ultimately decreased LDL-receptor-mediated cholesterol uptake by the liver [10].

Here we observed that systemic inflammation (CRP) and disease activity (DAS28-CRP) inversely associated with ABCA1-CEC. This is in agreement with earlier reports of low ABCA1-CEC function of inflammatory HDL in healthy volunteers treated with LPS [30]. We further demonstrated that ABCA1-CEC associated with greater baseline atherosclerosis burden in patients with above median CRP; in contrast, when CRP was lower, increasing ABCA1-CEC associated with lower atherosclerosis burden at baseline. Likewise, higher ABCA1-CEC was linked to fewer new calcified plaques in patients with below median time-averaged CRP during follow-up. These observations may be explained considering that low inflammation and good disease control are associated with the improvement of HDL composition, maturation and function, as well as with less cholesterol loading onto macrophages by LDL. Indeed, inflammation changes HDL composition, decreases its anti-oxidant activity and CEC, blocks LCAT and oxidizes LDL, promoting its ability to load cholesterol to cells through unregulated scavenger receptors [31,32].

Corticosteroids are commonly prescribed to patients with active disease or flares of RA. Indeed, prednisone use at baseline and exposure during follow-up associated with higher inflammation. As aforementioned, when inflammation is high, cholesterol loading on arterial wall macrophages by oxidized LDL increases and promotes atherosclerosis [31,32]. Moreover, the block in pre-β HDL maturation occurring in a proinflammatory environment, evidenced by enhanced ABCA1-CEC and depressed ABCG1-CEC, may impede the conclusion of the reverse cholesterol transport process, thus sustaining atherogenesis.

However, we previously showed that cumulative prednisone dose predicted coronary atherosclerosis progression in RA independently of inflammation [11]. Likewise in the present study, ABCA1-CEC associated with lower plaque progression in prednisone unexposed patients and greater plaque progression in those receiving higher weighted average prednisone doses independently of inflammation and ASCVD score; this suggests an independent effect of prednisone on cell cholesterol homeostasis. Indeed, we previously found that corticosteroids reduced cholesterol efflux to standard acceptors and promoted cholesterol loading and foam cell formation in human macrophages in vitro [33]. Taken together, these observations suggest that prednisone may in fact contribute to the proatherogenic environment it was intended to treat, by directly impacting cholesterol handling in arterial wall macrophages. In the absence of prednisone use and therefore in the context of lower inflammation, lower macrophage cholesterol uptake and higher expression of cholesterol transporters for efflux, higher ABCA1-CEC—i.e. well-functioning pre-β HDL—may efficiently attenuate atherosclerosis progression. This observation has significant clinical repercussions since ABCA1-CEC was linked to higher cardiovascular risk in patients using prednisone but not nonusers.

We showed that higher ABCA1-CEC associated with more fully calcified plaques at baseline in methotrexate nonusers but not in users. Methotrexate treatment decreased plaque burden and intimal macrophage migration in cholesterol-fed rabbits [34] and ApoE$^{-/-}$ high-fat-fed mice [35]. Uptake of methotrexate in plaque reduced regulated upon activation, normal T Cell expressed and secreted (RANTES), the chemokine responsible for immune cell migration and homing into the vessel wall [36]. Methotrexate also induces release of adenosine, which, upon binding adenosine A2A receptors on inflammatory cells, promotes intracellular c-AMP release [37]. This, in turn, upregulates ABCA1 expression and favors ABCA1-mediated cholesterol efflux; it also suppresses scavenger receptors CD36, SR-A1 and proinflammatory cytokines [36]. In peripheral blood mononuclear cells from patients with RA, methotrexate increased ABCA1 mRNA [38]. Methotrexate may therefore reduce atherosclerosis by facilitating outflow of cholesterol from the arterial wall and controlling local inflammation [36,38].

We also observed that ABCA1-CEC associated with greater atherosclerosis burden and progression in baseline bDMARD nonusers. In contrast, ABCA1-CEC associated with fewer new plaques in bDMARD users, independently of time-averaged CRP. Patients receiving bDMARDs typically have inadequate response to csDMARDs; bDMARD users in our cohort had longer RA duration, greater prednisone utilization and higher time-averaged disease activity during follow-up (not shown)—all factors associated with accelerated atherosclerosis and cardiovascular event risk [11,12]. Yet, bDMARDs independently attenuated cardiovascular risk and associated with lower lipid content and atherosclerotic plaque stabilization [14], enabling the beneficial effects of ABCA1-CEC on the atherosclerotic process. TNF-α promotes atherosclerosis by downregulating ABCA1 and G1 protein expression, which impairs reverse cholesterol transport, and upregulating CD36 and SR-A1, which enhances cholesterol uptake [39,40]. TNF-α also suppresses LCAT function [41]. Infliximab reversed TNF-α-mediated suppression of cholesterol transporters for efflux [40] and adalimumab reduced cholesterol loading by acting both on serum lipoproteins and directly on macrophages [19]. Furthermore, both TNF-α and non-TNF-α inhibitor bDMARDs were shown to restore LCAT function [42]. Collectively, these observations suggest that normalization of the various steps in cholesterol metabolism may lead to the unmasking of the protective effects of ABCA1-CEC and to attenuation of atherosclerosis progression.

Our study has certain limitations. First, analyses of the effects of ABCA1-CEC and its interactions with CRP and medications were not prespecified or powered for in our original study design. Hence, findings should be considered exploratory and prospectively validated. Secondly, we did not measure pre-β HDL particles or LCAT activity; however, the association between pre-β HDL levels and ABCA1-CEC and the role of LCAT on pre-β HDL maturation are extensively reported [23,24]. Lastly, patients found to have coronary plaque at screening had statin and/or aspirin treatments initiated regardless of clinical indication. Despite covarying for statin exposure, this may have still influenced atherosclerosis progression and cardiovascular risk [43], as well as the impact of ABCA1-CEC and moderators on these outcomes.

5. Conclusion

ABCA1-CEC associated with lower coronary plaque burden and progression in patients with low baseline and cumulative CRP, prednisone nonusers and baseline bDMARD users. In contrast, ABCA1-CEC associated with accelerated atherosclerosis in patients with high baseline CRP, prednisone exposure, and baseline methotrexate nonusers or bDMARDs nonusers. While ABCA1-CEC appears atheroprotective in well-treated RA with controlled disease activity, when RA is not controlled ABCA1-CEC may instead reflect a proatherogenic state promoted by inflammation.

Author contributions

George Karpouzas: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. Bianca Papotti: Investigation, Writing – Review & Editing. Sarah Ormseth: Formal analysis, Data Curation, Writing – Review & Editing, Visualization. Marcella Palumbo: Investigation, Writing – Review & Editing. Elizabeth Hernandez: Investigation, Data Curation, Writing – Review & Editing. Maria Pia Adorni: Investigation, Writing – Review & Editing. Francesca Zimetti: Investigation, Writing – Review & Editing. Matthew Budoff: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – Review & Editing. Nicoletta Ronda:

Conceptualization, Methodology Validation, Investigation, Resources, Supervision, Data Curation, Writing – Review & Editing. All authors critically revised the manuscript for important intellectual content and approved the final version to be published.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: George A Karpouzas reports financial support was provided by American Heart Association Inc. George A Karpouzas reports financial support was provided by Pfizer.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtauto.2023.100209.

References

[1] M.P. Adorni, N. Ronda, F. Bernini, F. Zimetti, High density lipoprotein cholesterol efflux capacity and atherosclerosis in cardiovascular disease: pathophysiological aspects and pharmacological perspectives, Cells 10 (2021) 574.
[2] D.J. Rader, E.T. Alexander, G.L. Weibel, J. Billheimer, G.H. Rothblat, The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis, J. Lipid Res. 50 (Suppl) (2009) S189–S194.
[3] T. Oshita, R. Toh, Y. Nagano, K. Kuroda, Y. Nagasawa, A. Harada, K. Murakami, M. Kiriyama, K. Yoshikawa, K. Miwa, T. Kubo, T. Iino, M. Nagao, Y. Irino, T. Hara, M. Shinohara, H. Otake, T. Shinke, K. Nakajima, T. Ishida, K.-I. Hirata, Association of cholesterol uptake capacity, a novel indicator for HDL functionality, and coronary plaque properties: an optical coherence tomography-based observational study, Clin. Chim. Acta 503 (2020) 136–144.
[4] M.T. Soria-Florido, H. Schröder, M. Grau, M. Fitó, C. Lassale, High density lipoprotein functionality and cardiovascular events and mortality: a systematic review and meta-analysis, Atherosclerosis 302 (2020) 36–42.
[5] B. Xie, J. He, Y. Liu, T. Liu, C. Liu, A meta-analysis of HDL cholesterol efflux capacity and concentration in patients with rheumatoid arthritis, Lipids Health Dis. 20 (2021) 18.
[6] B. Tejera-Segura, M. Macía-Díaz, J.D. Machado, A. de Vera-González, J.A. García-Dopico, J.M. Olmos, J.L. Hernández, F. Díaz-González, M.A. González-Gay, I. Ferraz-Amaro, HDL cholesterol efflux capacity in rheumatoid arthritis patients: contributing factors and relationship with subclinical atherosclerosis, Arthritis Res. Ther. 19 (2017) 113.
[7] N. Ronda, E. Favari, M.O. Borghi, F. Ingegnoli, M. Gerosa, C. Chighizola, F. Zimetti, M.P. Adorni, F. Bernini, P.L. Meroni, Impaired serum cholesterol efflux capacity in rheumatoid arthritis and systemic lupus erythematosus, Ann. Rheum. Dis. 73 (2014) 609–615.
[8] D. Greco, R. Gualtierotti, P. Agosti, M.P. Adorni, F. Ingegnoli, M. Rota, F. Bernini, P.L. Meroni, N. Ronda, Anti-atherogenic modification of serum lipoprotein function in patients with rheumatoid arthritis after tocilizumab treatment, a pilot study, J. Clin. Med. 9 (2020).
[9] G.A. Karpouzas, B. Papotti, S.R. Ormseth, M. Palumbo, E. Hernandez, M.P. Adorni, F. Zimetti, M.J. Budoff, N. Ronda, ATP-binding cassette G1 membrane transporter-mediated cholesterol efflux capacity influences coronary atherosclerosis and cardiovascular risk in rheumatoid arthritis, J. Autoimmun. 136 (2023), 103029.
[10] G.A. Karpouzas, B. Papotti, S.R. Ormseth, M. Palumbo, E. Hernandez, C. Marchi, M. P. Adorni, F. Zimetti, M. Budoff, N. Ronda, Statins influence the relationship between ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux and coronary atherosclerosis in rheumatoid arthritis [abstract], Ann. Rheum. Dis. 82 (2023) 419.
[11] G.A. Karpouzas, S.R. Ormseth, E. Hernandez, M.J. Budoff, Impact of cumulative inflammation, cardiac risk factors, and medication exposure on coronary atherosclerosis progression in rheumatoid arthritis, Arthritis Rheumatol. 72 (2020) 400–408.
[12] A.J. Ocon, G. Reed, D.A. Pappas, J.R. Curtis, J.M. Kremer, Short-term dose and duration-dependent glucocorticoid risk for cardiovascular events in glucocorticoid-naive patients with rheumatoid arthritis, Ann. Rheum. Dis. 80 (2021) 1522–1529.
[13] S. Suissa, S. Bernatsky, M. Hudson, Antirheumatic drug use and the risk of acute myocardial infarction, Arthritis Rheum. 55 (2006) 531–536.
[14] G.A. Karpouzas, S.R. Ormseth, E. Hernandez, M.J. Budoff, Biologics may prevent cardiovascular events in rheumatoid arthritis by Inhibiting coronary plaque formation and stabilizing high-risk lesions, Arthritis Rheumatol. 72 (2020) 1467–1475.
[15] G.A. Karpouzas, J. Malpeso, T.-Y. Choi, D. Li, S. Munoz, M.J. Budoff, Prevalence, extent and composition of coronary plaque in patients with rheumatoid arthritis without symptoms or prior diagnosis of coronary artery disease, Ann. Rheum. Dis. 73 (2014) 1797–1804.
[16] A.S. Agatston, W.R. Janowitz, F.J. Hildner, N.R. Zusmer, M. Viamonte, R. Detrano, Quantification of coronary artery calcium using ultrafast computed tomography, J. Am. Coll. Cardiol. 15 (1990) 827–832.
[17] J. Leipsic, S. Abbara, S. Achenbach, R. Cury, J.P. Earls, G.J. Mancini, K. Nieman, G. Pontone, G.L. Raff, SCCT guidelines for the interpretation and reporting of coronary CT angiography: a report of the Society of Cardiovascular Computed Tomography Guidelines Committee, J Cardiovasc Comput Tomogr 8 (2014) 342–358.
[18] M.J. Peters, D.P. Symmons, D. McCarey, B. Dijkmans, P. Nicola, T.K. Kvien, I. B. McInnes, H. Haentzschel, M. Gonzalez-Gay, S. Provan, A. Semb, P. Sidiropoulos, G. Kitas, Y.M. Smulders, M. Soubrier, Z. Szekanecz, N. Sattar, M.T. Nurmohamed, EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis, Ann. Rheum. Dis. 69 (2010) 325–331.
[19] N. Ronda, D. Greco, M.P. Adorni, F. Zimetti, E. Favari, G. Hjeltnes, K. Mikkelsen, M.O. Borghi, E.G. Favalli, R. Gatti, I. Hollan, P.L. Meroni, F. Bernini, Newly identified antiatherosclerotic activity of methotrexate and adalimumab: complementary effects on lipoprotein function and macrophage cholesterol metabolism, Arthritis Rheumatol. 67 (2015) 1155–1164.
[20] B.F. Asztalos, K.V. Horvath, K. Kajinami, C. Nartsupha, C.E. Cox, M. Batista, E. J. Schaefer, A. Inazu, H. Mabuchi, Apolipoprotein composition of HDL in cholesteryl ester transfer protein deficiency, J. Lipid Res. 45 (2004) 448–455.
[21] D.C. Goff, D.M. Lloyd-Jones, G. Bennett, S. Coady, R.B. D'Agostino, R. Gibbons, P. Greenland, D.T. Lackland, D. Levy, C.J. O'Donnell, J.G. Robinson, J.S. Schwartz, S.T. Shero, S.C. Smith, P. Sorlie, N.J. Stone, P.W.F. Wilson, H.S. Jordan, L. Nevo, J. Wnek, J.L. Anderson, J.L. Halperin, N.M. Albert, B. Bozkurt, R.G. Brindis, L. H. Curtis, D. DeMets, J.S. Hochman, R.J. Kovacs, E.M. Ohman, S.J. Pressler, F. W. Sellke, W.-K. Shen, S.C. Smith, G.F. Tomaselli, American college of cardiology/American heart association task force on practice guidelines, 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American college of cardiology/American heart association task force on practice guidelines, Circulation 129 (2014) S49–S73.
[22] A. Nichol, M. Bailey, M. Egi, V. Pettila, C. French, E. Stachowski, M.C. Reade, D. J. Cooper, R. Bellomo, Dynamic lactate indices as predictors of outcome in critically ill patients, Crit. Care 15 (2011) R242.
[23] M. de la Llera-Moya, D. Drazul-Schrader, B.F. Asztalos, M. Cuchel, D.J. Rader, G. H. Rothblat, The ability to promote efflux via ABCA1 determines the capacity of serum specimens with similar high-density lipoprotein cholesterol to remove cholesterol from macrophages, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 796–801.
[24] L. Calabresi, E. Favari, E. Moleri, M.P. Adorni, M. Pedrelli, S. Costa, W. Jessup, I. C. Gelissen, P.T. Kovanen, F. Bernini, G. Franceschini, Functional LCAT is not required for macrophage cholesterol efflux to human serum, Atherosclerosis 204 (2009) 141–146.
[25] B.F. Asztalos, K.V. Horvath, E.J. Schaefer, High-density lipoprotein particles, cell-cholesterol efflux, and coronary heart disease risk, Arterioscler. Thromb. Vasc. Biol. 38 (2018) 2007. –15.
[26] C. Charles-Schoeman, J. Watanabe, Y.Y. Lee, D.E. Furst, S. Amjadi, D. Elashoff, G. Park, M. McMahon, H.E. Paulus, A.M. Fogelman, S.T. Reddy, Abnormal function of high-density lipoprotein is associated with poor disease control and an altered protein cargo in rheumatoid arthritis, Arthritis Rheum. 60 (2009) 2870–2879.
[27] C. Charles-Schoeman, R. Fleischmann, J. Davignon, H. Schwartz, S.M. Turner, C. Beysen, M. Milad, M.K. Hellerstein, Z. Luo, I.V. Kaplan, R. Riese, A. Zuckerman, I.B. McInnes, Potential mechanisms leading to the abnormal lipid profile in patients with rheumatoid arthritis versus healthy volunteers and reversal by tofacitinib, Arthritis Rheumatol. 67 (2015) 616–625.
[28] M. Koseki, S. Yamashita, M. Ogura, Y. Ishigaki, K. Ono, K. Tsukamoto, M. Hori, K. Matsuki, S. Yokoyama, M. Harada-Shiba, Current diagnosis and management of tangier disease, J Atheroscler Thromb 28 (2021) 802–810.
[29] R. Duivenvoorden, A.G. Holleboom, B. van den Bogaard, A.J. Nederveen, E. de Groot, B.A. Hutten, A.W. Schimmel, G.K. Hovingh, J.J.P. Kastelein, J. A. Kuivenhoven, E.S.G. Stroes, Carriers of lecithin cholesterol acyltransferase gene mutations have accelerated atherogenesis as assessed by carotid 3.0-T magnetic resonance imaging, J. Am. Coll. Cardiol. 58 (2011) 2481–2487.
[30] M. de la Llera Moya, F.C. McGillicuddy, C.C. Hinkle, M. Byrne, M.R. Joshi, V. Nguyen, J. Tabita-Martinez, M.L. Wolfe, K. Badellino, L. Pruscino, N.N. Mehta,

B.F. Asztalos, M.P. Reilly, Inflammation modulates human HDL composition and function in vivo, Atherosclerosis 222 (2012) 390–394.
[31] G.A. Karpouzas, B. Papotti, S.R. Ormseth, M. Palumbo, E. Hernandez, C. Marchi, F. Zimetti, M.J. Budoff, N. Ronda, Serum cholesterol loading capacity on macrophages is regulated by seropositivity and C-reactive protein in rheumatoid arthritis patients, Rheumatology 62 (2023) 1254–1263.
[32] G.A. Karpouzas, B. Papotti, S. Ormseth, M. Palumbo, E. Hernandez, M.P. Adorni, F. Zimetti, M. Budoff, N. Ronda, Serum cholesterol loading capacity on macrophages is linked to coronary atherosclerosis and cardiovascular event risk in rheumatoid arthritis, RMD Open 8 (2022), e002411.
[33] D. Greco, E. Favari, M.P. Adorni, F. Zimetti, R. Gatti, F. Bernini, N. Ronda, Hydrocortisone directly promotes cholesterol accumulation in macrophages, Ann. Rheum. Dis. 73 (2014) 1274–1276.
[34] A. Bulgarelli, A.A. Martins Dias, B. Caramelli, R.C. Maranhão, Treatment with methotrexate inhibits atherogenesis in cholesterol-fed rabbits, J. Cardiovasc. Pharmacol. 59 (2012) 308–314.
[35] C. Stigliano, M.R. Ramirez, J.V. Singh, S. Aryal, J. Key, E. Blanco, P. Decuzzi, Methotraxate-loaded hybrid nanoconstructs target vascular lesions and inhibit atherosclerosis progression in ApoE-/- mice, Adv Healthc Mater 6 (2017), 1601286.
[36] V. Di Francesco, D. Gurgone, R. Palomba, d Ferreira, T. Catelani, A. Cervadoro, P. Maffia, P. Decuzzi, Modulating lipoprotein transcellular transport and atherosclerotic plaque formation in ApoE-/- mice via nanoformulated lipid-methotrexate conjugates, ACS Appl. Mater. Interfaces 12 (2020) 37943–37956.
[37] M. Cutolo, A. Sulli, C. Pizzorni, B. Seriolo, R.H. Straub, Anti-inflammatory mechanisms of methotrexate in rheumatoid arthritis, Ann. Rheum. Dis. 60 (2001) 729–735.
[38] A.B. Reiss, S.E. Carsons, K. Anwar, S. Rao, S.D. Edelman, H. Zhang, P. Fernandez, B.N. Cronstein, E.S.L. Chan, Atheroprotective effects of methotrexate on reverse cholesterol transport proteins and foam cell transformation in human THP-1 monocyte/macrophages, Arthritis Rheum. 58 (2008) 3675–3683.
[39] A.Z.S. Ma, Q. Zhang, Z.Y. Song, TNFa alter cholesterol metabolism in human macrophages via PKC-θ-dependent pathway, BMC Biochem. 14 (2013) 20.
[40] I. Voloshyna, S. Seshadri, K. Anwar, M.J. Littlefield, E. Belilos, S.E. Carsons, A. B. Reiss, Infliximab reverses suppression of cholesterol efflux proteins by TNF-α: a possible mechanism for modulation of atherogenesis, BioMed Res. Int. 2014 (2014), 312647.
[41] H. Ly, O.L. Francone, C.J. Fielding, J.K. Shigenaga, A.H. Moser, C. Grunfeld, K. R. Feingold, Endotoxin and TNF lead to reduced plasma LCAT activity and decreased hepatic LCAT mRNA levels in Syrian hamsters, J. Lipid Res. 36 (1995) 1254–1263.
[42] A.B. Márquez, S. Nazir, E.P.C. van der Vorst, High-density lipoprotein modifications: a pathological consequence or cause of disease progression? Biomedicines 8 (2020) 549.
[43] G.A. Karpouzas, S.R. Ormseth, E. Hernandez, M.J. Budoff, The impact of statins on coronary atherosclerosis progression and long-term cardiovascular disease risk in rheumatoid arthritis, Rheumatology 61 (2022) 1857–1866.