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Cholesterol efflux by high density lipoproteins is impaired in patients with active rheumatoid arthritis

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Abstract

Objectives—Reverse cholesterol transport (RCT) is a major antiatherogenic function of high density lipoprotein (HDL). In the current work, the authors evaluated whether the RCT capacity of HDL from rheumatoid arthritis (RA) patients is impaired when compared to healthy controls.

Methods—HDL was isolated from 40 patients with RA and 40 age and sex matched healthy controls. Assays of cholesterol efflux, HDL's antioxidant function and paraoxanase-1 (PON-1) activity were performed as described previously. Plasma myeloperoxidase (MPO) activity was assessed by a commercially available assay.

Results—Mean cholesterol efflux capacity of HDL was not significantly different between RA patients ($40.2\% \pm 11.1\%$) and controls ($39.5\% \pm 8.9\%$); $p=0.75$. However, HDL from RA patients with high disease activity measured by a disease activity score using 28 joint count ($\text{DAS28} > 5.1$), had significantly decreased ability to promote cholesterol efflux compared to HDL from patients with very low disease activity/clinical remission ($\text{DAS28} < 2.6$). Significant correlations were noted between cholesterol efflux and the DAS28 ($r=-0.39$, $p=0.01$) and erythrocyte sedimentation rate, ($r=-0.41$, $p=0.0009$). Higher plasma MPO activity was associated with worse HDL function ($r=0.41$, $p=0.009$ (antioxidant capacity); $r=0.35$, $p=0.03$ (efflux)). HDL's ability to promote cholesterol efflux was modestly but significantly correlated with its antioxidant function ($r=-0.34$, $p=0.03$).

Conclusions—The cholesterol efflux capacity of HDL is impaired in RA patients with high disease activity and is correlated with systemic inflammation and HDL's antioxidant capacity. Attenuation of HDL function, independent of HDL cholesterol levels, may suggest a mechanism by which active RA contributes to increased cardiovascular (CV) risk.

INTRODUCTION

Reverse cholesterol transport (RCT) is a multi-step process resulting in the net movement of cholesterol via high density lipoprotein (HDL) from peripheral tissues, including foam cells, back to the liver for excretion.¹ Promotion of cholesterol efflux by HDL is considered a major antiatherogenic function of HDL.^{2,3} Recent work has proposed that inflammation may impair RCT.⁴

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Rheumatoid arthritis (RA) is a systemic autoimmune disease associated with inflammatory arthritis and a significantly increased atherosclerotic risk.⁵ Active RA and high levels of systemic inflammation have been associated with increased cardiovascular disease (CVD).⁶⁷ Additional understanding of the mechanisms through which RA-induced inflammation increases atherosclerotic risk is needed for the development of effective CV risk reduction treatments and strategies in the RA population.

Previous work by our group has suggested that the antioxidant function of HDL is abnormal in patients with RA, and is correlated with disease activity.⁸ We have also described an altered protein cargo associated with HDL with abnormal antioxidant capacity.⁸ Popa *et al* reported increases in paraoxonase-1 (PON-1) activity and an improvement in HDL's antioxidant capacity following tumour necrosis factor α (TNF α) inhibitor therapy.⁹ The current work evaluates whether the RCT function of HDL from RA patients is also impaired when compared to healthy controls, and whether it is associated with systemic inflammation and alterations in the plasma activity of myeloperoxidase (MPO), a heme peroxidase abundant in activated neutrophils and previously linked to impairment in RCT.¹⁰¹¹

PATIENTS AND METHODS

Human subjects

RA patients were recruited from the rheumatology offices at the University of California, Los Angeles (UCLA) via flyers posted in the offices and in the UCLA Medical Center. All RA patients met the American College of Rheumatology criteria for RA, which was verified by chart review. Age, sex and ethnicity matched healthy controls were recruited via flyers in the UCLA community. All subjects gave written informed consent for the study under a protocol approved by the Human Research Subject Protection Committee at UCLA.

On the day of the study visit, all patients had assessments of inflammatory markers including high sensitivity C reactive protein (HSCRP) and Westergren erythrocyte sedimentation rate erythrocyte sedimentation rate (ESR), and fasting lipid profiles. Disease activity in RA patients was assessed by 28 tender and swollen joint counts and patient/physician global assessments on a visual analogue scale; 0–100. A disease activity scale using the 28 joint count (DAS28) was calculated for each patient.

For the HDL antioxidant function, cholesterol efflux, MPO, PON-1, and apolipoprotein A-I (apoA-I) studies, blood was collected in heparinized tubes (Becton Dickinson) and 50% sucrose solution was added at a ratio of 1 volume sucrose to 4 volumes of plasma,¹² thoroughly mixed, divided into aliquots and kept frozen at -80°C until use.

Evaluation of HDL's antioxidant function by the cell free assay (CFA)

The CFA was a modification of a previously published method¹² using low density lipoprotein (LDL) as the fluorescence-inducing agent. Control LDL was prepared as described previously.¹² HDL-containing supernatants were first isolated by the dextran sulphate method. 50 μl of HDL magnetic bead reagent, (Polymedco catalog #5030), were mixed with 250 μl of patient plasma and incubated for 5 min at room temperature. The solution was then incubated for an additional 5 min on a magnetic particle concentrator. HDL-cholesterol in the supernatant was quantified using a standard assay (Thermo DMA Co, San Jose, California, USA).

To determine the anti-inflammatory properties of HDL, the change in fluorescence intensity as a result of the oxidation of dihydrodichlorofluorescein (DCFH) in incubations with a standard normal control LDL in the absence or presence of the test HDL was assessed. Dihydrodichlorofluorescein diacetate (DCFH-DA) (Molecular Probes, Inc) was first

dissolved in fresh methanol at 2.0 mg/ml and incubated in the dark at room temperature for 20 min, resulting in release of DCFH. 25 µl of LDL-cholesterol (100 µg/ml) was mixed with 50 µl of test HDL (100 µg HDL-cholesterol/ml) in black, flat bottom polystyrene micro-titer plates and incubated at 37°C with rotation for 30 min. 25 µl of DCFH solution (0.2 mg/ml) was then added to each well, mixed and incubated at 37°C for 1 h with rotation. Fluorescence was determined with a plate reader (Spectra Max, Gemini XS; Molecular Devices) at an excitation wavelength of 485 nm, emission wavelength of 530 nm, and cut-off of 515 nm with photomultiplier sensitivity set at medium. Values for intra- and interassay variability were 0.5±0.37% and 3.0±1.7%, respectively.¹³

HDL-mediated cellular cholesterol efflux

Assays of cellular cholesterol efflux were performed as previously described¹⁴ with minor modifications. In brief, mouse macrophage RAW264.7 cells (ATCC, Manassas, Virginia, USA) were cultured on 24-well tissue culture plates and grown in DMEM media (GIBCO-BRL, Grand Island, New York, USA) with 10% FBS overnight. The cells were washed and loaded with ³H-cholesterol (0.33 µCi/ml) and acetylated LDL (50 µg/ml) in DMEM media supplemented with 50 mM glucose, 2 mM glutamine and 0.2% fatty acid free bovine serum albumin (BSA) (DGGB) (Sigma, St Louis, Missouri, USA) overnight to allow cell cholesterol pools to equilibrate. Cells were washed twice and incubated overnight with DGGB+0.1 mM Br-cAmp. Cholesterol efflux from macrophages by HDL was determined by incubating the test HDL @ 25 µg/ml, (isolated from plasma by dextran bead method as above), in DGGB with labelled cells for 4 h at 37°C. Radioactivity in the supernatants and total cell extracts were measured and expressed as the percentage of total radioactive counts removed from the cells by HDL during the efflux period.

Assessment of plasma MPO activity

The activity of MPO was measured in plasma using the InnoZyme MPO activity assay kit (EMD Chemicals, Darmstadt, Germany).

In brief, patient plasma was added to a 96-well plate with an immobilised polyclonal antibody specific for human MPO. Activity of captured MPO was measured using a detection reagent that includes (tetramethyl benzidine) TMB and hydrogen peroxide. Following colour development, the reaction was stopped with sulphuric acid and the absorbance of the oxidised TMB detected at 450 nm.

Assessment of PON-1 activity

Paraoxonase/arylesterase activity was measured with phenylacetate as a substrate as described previously with minor modifications.¹⁵ Plasma was diluted at 1:50 dilution. Briefly, samples were incubated with 25 ml of phenylacetate (Sigma Cat# P-2396, 100 ml) in 50 ml of assay buffer containing 9.0 mM Tris.HCl, pH 8.0 and 0.9 mM CaCl₂. The increase in absorbance was continuously recorded at 270 nm with total time of 2 min and interval time of 15 s. Enzyme activity was calculated per ml of plasma using the molar extinction coefficient of 1.310 M⁻¹ cm⁻¹.

Assessment of ApoA-I

ApoA-I levels were determined by direct ELISA according to the manufacturer's protocol (AssayPro, St Charles, Missouri, USA). In brief, 25 µl of standard or sample were added to a 96-well plate coated with a polyclonal antibody against human Apo A-I. 25 µl of biotinylated ApoA-I was added to each well and the plate was incubated for 2 h, washed and 50 µl of streptavidin-peroxidase conjugate was added for 30 min. 50 µl of chromogen

substrate was added per well and incubated for 10 min followed by 50 μ l of stop solution. Absorbance was read on a microplate reader at a wavelength of 450 nm.

Statistical analysis

Data were analysed using JMP IN 8.0 (SAS Institute Inc, Cary, North California, USA). Patient groups were compared using student's t test for continuous variables and the χ^2 test of association for categorical variables, along with Fisher's exact test for small sample sizes. When needed, non-parametric Wilcoxon rank-sum tests were used to analyse continuous variables. The significance level was prespecified at $p < 0.05$.

Correlations between variables were evaluated using the Pearson's correlation coefficient for normally distributed data and Spearman's correlation coefficient for non-parametric data. Forward stepwise linear regression analysis was done to confirm an association of systemic inflammation measured by ESR with cholesterol efflux. Covariates included ESR, diabetes, smoking and prednisone use. Forward stepwise linear regression analysis was also performed to confirm an association of systemic inflammation with MPO activity in RA. Covariates included measures of inflammation and demographic factors (age and sex).

RESULTS

No difference in cholesterol efflux between RA patients and age and sex matched healthy controls

Forty patients with RA were compared to forty age, sex and ethnicity matched healthy controls. The capacity of isolated HDL to promote cholesterol efflux from macrophages in vitro was not different between the two groups (table 2). Significant but modest differences in inflammatory markers were noted between the two groups (table 2). Diabetes and hypertension were increased in the RA group, but no significant differences in mean apoA-I, HDL, LDL, or total cholesterol levels were noted (tables 1–2). Additional RA specific clinical data including disease modifying drug use are provided in table 3.

Cholesterol efflux is significantly decreased in RA patients with high disease activity

HDL from RA patients with high disease activity, ($\text{DAS28} > 5.1$) ($n=18$), had significantly decreased ability to promote cholesterol efflux compared to HDL from patients with very low disease activity/clinical remission ($\text{DAS28} < 2.6$) ($n=7$) (figure 1A). No significant differences in age, sex, traditional CV risk factors or RA disease specific characteristics were observed between the high and low disease activity groups, however, there were trends for longer disease duration, increased smoking, diabetes and prednisone use in the high disease activity group (tables 4, 5).

A significant correlation was also noted between cholesterol efflux and disease activity measured by the DAS28 in all RA patients ($r = -0.39$, $p = 0.01$). Higher RA disease activity was associated with decreased efflux by HDL. A similar correlation was observed with ESR, ($r = -0.41$, $p = 0.009$), and a trend noted with HSCRP ($r = -0.29$, $p = 0.08$). Multivariate linear regression analysis was performed to adjust for smoking, diabetes, and prednisone use as potential confounders, and ESR remained significantly associated with cholesterol efflux (table 6).

Correlation between HDL-mediated cholesterol efflux and HDL's antioxidant function

HDL's ability to promote cholesterol efflux showed a modest but significant correlation with its ability to inhibit oxidation ($r = -0.34$, $p = 0.03$) in patients with RA. Worse antioxidant function of HDL, (a higher HII), was associated with lower cholesterol efflux from macrophages by HDL.

MPO activity is increased in patients with RA compared to healthy controls

Plasma MPO activity was significantly higher in patients with RA compared to controls (figure 2). MPO activity was significantly correlated with RA disease activity measured by the DAS28 ($r=0.45$, $p=0.004$). Higher RA disease activity was associated with higher MPO activity. Significant differences were also noted in patients with high disease activity versus low disease activity defined by the DAS28 (figure 1B). MPO activity was significantly correlated with levels of systemic inflammation measured by HSCRP, ($r=0.49$; $p=0.002$) and the erythrocyte sedimentation rate (ESR), ($r=0.39$; $p=0.01$) in patients with RA. Linear regression analysis confirmed the association of systemic inflammation with MPO activity in RA patients while controlling for basic demographic factors (table 7).

MPO activity is associated with abnormal cholesterol efflux and antioxidant function of HDL

Cholesterol efflux by HDL showed a significant correlation with MPO activity in patients with RA. Higher plasma MPO activity was associated with lower cholesterol efflux by HDL ($r=-0.35$, $p=0.03$). Patients with the highest quartile of plasma MPO activity had significantly decreased HDL-mediated cholesterol efflux compared to patients with the lowest quartile of MPO activity ($34\pm15\%$ and $47\pm7\%$, respectively); $p=0.03$. No significant differences in age, sex, or traditional lipoprotein levels were noted between the patient groups (data not shown).

HDL's antioxidant function was decreased in RA patients compared to matched controls as shown by a higher HDL inflammatory index (HII) (figure 2). MPO activity was significantly correlated with HDL's antioxidant function ($r=-0.41$; $p=0.0009$) in patients with RA; higher plasma MPO activity was associated with worse HDL function. HDL function was also significantly correlated with RA disease activity measured by the DAS28 ($r=-0.42$, $p=0.007$). Higher RA disease activity was associated with higher MPO activity and worse HDL function.

PON-1 activity is lower in RA patients and correlates with the antioxidant function of HDL

The activity of PON-1, an antioxidant enzyme associated with HDL, was lower in RA patients compared to healthy controls ($p=0.03$) (figure 2). PON-1 activity was significantly correlated with HDL's antioxidant function ($r=-0.38$, $p=0.0005$) in RA patients and controls. Higher PON-1 activity was associated with a lower HII and better HDL antioxidant function.

DISCUSSION

RA is a systemic autoimmune disease associated with chronic inflammation and oxidative stress.¹⁶ Patients with RA have a known increased atherosclerotic risk including increased risk of sudden death, which is not fully explained by traditional coronary risk factors.^{17,18} Previous work in RA patients has linked the increase in CVD to an increased inflammatory burden.⁶⁷

Introduction of new disease-modifying antirheumatic drugs, has significantly improved the quality of life and functioning for many RA patients.¹⁹ Recent evidence also suggests potential CV protective effects of biologic therapies such as TNF α inhibitors.²⁰ Poppa *et al* previously reported that TNF inhibition increases PON-1 activity and improves HDL's antioxidant function.⁹ However, despite recent advances, evidence from population-based studies of inception cohorts also suggests that survival in RA patients has not improved,²¹ and many RA patients are not able to achieve or maintain optimal disease control. Better

understanding of the mechanisms which link active RA to increased atherosclerotic risk is important to the development of targeted CV therapeutics and risk reduction strategies.

In the current work, we evaluated whether the RCT capacity of HDL from RA patients is impaired when compared to healthy controls. Differences in cholesterol efflux were not found between patients and healthy controls. However, significant differences were noted between RA patients with low disease activity/clinical remission (DAS28<2.6) compared to RA patients with high disease activity (DAS28>5.1). Significant correlations were also found between the ability of patient HDL to efflux cholesterol from macrophages in vitro and RA disease activity and systemic inflammation measured by ESR. The association of ESR with cholesterol efflux remained significant after adjustment in multivariate analysis for a limited number of confounders including diabetes, smoking, and prednisone use.

Inflammation has previously been proposed to impair RCT.⁴ McGillicuddy *et al* provided evidence that inflammation impedes several components of RCT in a mouse model of endotoxemia. In this model, lipopolysaccharide administration reduced cholesterol movement from macrophages to plasma as well as reduced cholesterol associated with HDL. Humans were evaluated following intravenous injection of endotoxin and cholesterol efflux was significantly decreased following onset of the inflammatory response. In this work, significant decreases in cholesterol efflux from baseline were noted when levels of CRP were in the range of 40 mg/dl (~400 mg/l HSCR).⁴ We hypothesise that differences in cholesterol efflux between RA patients and matched controls were not seen in our work due to relatively small differences in mean inflammatory markers between the groups, normal assay variability and a relatively small sample size. However, when patients with high disease activity were compared to patients with low disease activity, significant differences in cholesterol efflux were apparent. In addition, significant correlations were noted between cholesterol efflux and disease activity/systemic inflammation (ESR) as continuous variables. Higher levels of disease activity/systemic inflammation were associated with lower cholesterol efflux. This association remained in limited multivariate analysis as described above. Interestingly, significant effects of inflammation on CV events in RA patients have also been noted with very high levels of systemic inflammation measured by ESR.⁷

Myeloperoxidase is a protein abundant in the granules of neutrophils and to a lesser degree monocytes which generates reactive oxygen species to kill invading pathogens as part of the host defense.²² Previous work has demonstrated that many of the specific oxidation products generated by MPO are significantly enriched in the atherosclerotic plaque.²³ MPO is also increased in culprit lesions of patients with sudden cardiac death and MPO levels have been associated with the prevalence of coronary artery disease in the general population.^{23,24}

Plasma MPO activity was significantly increased in RA patients compared to age, ethnicity and sex matched healthy controls. MPO activity was also significantly correlated with disease activity measured by the DAS28, as well as with systemic inflammatory markers including ESR and HSCR. Higher disease activity and systemic inflammation were associated with higher plasma MPO activity. This data is consistent with a small study by Feijóo *et al* in which 10 RA patients had increased concentrations of MPO associated with active disease which were improved with infliximab therapy.²⁵

Work by Zheng *et al* first discovered that MPO binds to HDL via a specific binding domain on apoA-I, promoting selective targeting of the lipoprotein for oxidative modification and resultant loss of anti-inflammatory and cholesterol efflux functions.¹⁰¹¹ Additional studies have confirmed these observations^{26,27} as well as shown that site-specific oxidative modification of apoA-I within HDL inhibits specific enzymatic processes involved in RCT.^{28,29} We previously reported that MPO was elevated in HDL from a small number of

RA patients with active disease whose HDL was unable to inhibit oxidation of LDL in vitro.⁸ The current work shows that systemic MPO activity is significantly increased in patients with RA compared to controls, is associated with systemic inflammation, and is associated with both impairment in the antioxidant function of HDL as well as impairment in cholesterol efflux by HDL. This data suggests a pathway by which increased inflammation and oxidative stress in RA increases atherosclerotic risk.

The current study was not designed to assess associations of MPO activity or HDL function with CVD in patients with RA. However, interesting work by Dessein *et al* reported a significant association of the number of neutrophils, the predominant cell type producing MPO, and subclinical atherosclerosis in RA patients.³⁰ In addition, antibodies against apoA-I, the major site for oxidative modification of HDL by MPO which impairs cholesterol efflux, have been shown to predict major CV events in patients with RA.³¹ These associations as well as strong links of MPO with CV events in the general population²⁴ and consideration of RCT as a critical mechanism by which HDL exerts a protective effect on the development of atherosclerosis,²³ suggest that further work evaluating MPO and HDL function as potential mechanisms for increased CVD in RA patients may be warranted. To our knowledge, this is the first work to evaluate cholesterol efflux by HDL isolated from patients with RA in an in vitro assay.

The current study has some limitations. The major limitation of this study is the relatively small sample size that is subject to selection and referral bias. In addition, while multivariate analyses suggested an association of systemic inflammation with impaired cholesterol efflux while controlling for potential confounders including diabetes, smoking, and prednisone use, larger data sets are required to more fully assess the potential effects of traditional CV risk factors as well as RA disease characteristics on cholesterol efflux. There was a non-significant difference of 6 mg/dl in HDL-C levels between high and low disease activity groups and given previous work suggesting a decrease in HDL-C with high RA disease activity, this may also warrant further evaluation in larger cohorts. The current study is pilot in nature and the data must be considered hypothesis generating with further work warranted in larger RA cohorts. In addition, future studies may include evaluation of associations of MPO activity and HDL function including cholesterol efflux with CV outcomes and measures of subclinical atherosclerosis in RA. Investigation of the effects of RA therapeutics on these markers may be warranted. In conclusion, better understanding of the mechanisms underlying the link between 'RA inflammation' and CV risk is important so that appropriate therapeutics and prevention strategies can be developed.

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Figure 1. HDL-mediated cholesterol efflux and plasma MPO activity in RA patients with DAS28<2.6 (low disease activity/clinical remission) compared to RA patients with DAS28>5.1 (high disease activity).

Figure 2. MPO activity, HDL antioxidant function and paraoxonase-1 activity in patients with RA compared to healthy controls. (A) Plasma MPO activity, (B) HDL antioxidant function (measured by the HDL inflammatory index (HII)) and (C) paraoxonase (PON-1) activity.

Table 1

Clinical characteristics of patients with RA compared to age and sex matched healthy controls

Group	Age (years)	Ethnicity		HTN (%)	FH (%)	DM (%)	TOB (%)	Statin use (%)	BMI
		F (%)	% Caucasian						
RA (n=40)	56±12	85	50	35 [*]	13	18 [*]	5	18	28±7
Control (n=40)	52±13	80	63	15	8	0	5	8	25±4

^{*} p Value<0.05 compared to controls.

BMI, body mass index; DM, diabetes mellitus; F, female; FH, family History of a first degree relative with premature cardiovascular disease; HTN, hypertension; RA, rheumatoid arthritis; TOB, current smoker.

Table 2
Laboratory characteristics of patients with RA compared to age and sex matched healthy controls

Group	HSCR ^P (mg/l)	ESR (mm/h)	Cholesterol efflux	HDL-C (mg/dl)	LDL-C (mg/dl)	Total-C (mg/dl)	ApoA-I (ug/ml)
RA (n=40)	5.7±8.7 *	28±26 *	40.2%±11.1%	60±20	103±37	187±43	209±109
Control (n=40)	1.8±2.5	14±10	39.5%±8.9%	59±18	114±35	197±44	190±77

* p Value<0.05 compared to controls.

ApoA-I, apolipoprotein A-I; ESR, erythrocyte sedimentation rate; HDL-C, high density lipoprotein cholesterol; HSCR^P, high sensitivity C reactive protein; LDL-C, low density lipoprotein cholesterol; RA, rheumatoid arthritis; Total-C, total cholesterol.

Table 3

Disease characteristics of rheumatoid arthritis patients

Mean DAS28	4.68±1.86
Median DAS28	4.65±3.44
Disease duration	12.8±9.6
TNF-inhibitor (% yes)	35%
Methotrexate (% yes)	75%
Other Biologic (% yes)	20%
Other non-biologic DMARD (% yes)	23%
Prednisone use (% yes)	23%

DAS28, disease activity score using a 28 joint count (median DAS28±IQR, mean DAS28±SD); DMARD, disease-modifying antirheumatic drug; TNF, tumour necrosis factor.

Clinical characteristics of RA patients with high versus low disease activity/clinical remission defined by the DAS28

Table 4

Group	Age	F (%)	HSCRP (mg/l)	ESR (mm/hr)	HDL-C (mg/dl)	LDL-C (mg/dl)	HTN (%)	FH (%)	DM (%)	TOB (%)	STAT (%)	BMI
DAS28>5.1 (n=18)	58±12	83	10.1±10.5 *	47±26 *	60±16	114±44	47	18	29	12	28	29±8
DAS28<2.6 (n=7)	53±9	100	0.6±0.7	8±7	66±35	114±23	43	14	14	0	0	26±6

* p Value<0.05 compared to DAS28<2.6 group.

BMI, body mass index; DAS28, disease activity score using 28 joint count; DM, diabetes mellitus; ESR, erythrocyte sedimentation rate; F, female; FH, family history of a first degree relative with premature cardiovascular disease; HDL-C, high density lipoprotein cholesterol; HSCRP, high sensitivity C reactive protein; HTN, hypertension; LDL-C, low density lipoprotein cholesterol; STAT, statin; TOB, current smoker.

Table 5

Disease specific characteristics of rheumatoid arthritis patients with high versus low disease activity/clinical remission defined by the DAS28

Group	DAS28	Disease duration	TNFI (% yes)	MTX (% yes)	Other biologic (% yes)	Other non-biologic DMD (% yes)	Prednisone (% yes)
DAS28>5.1 (n=18)	6.4±0.8*	16±11	39	61	17	28	44
DAS28<2.6 (n=7)	2.1±0.4	8±7	29	71	14	29	0

* p Value<0.05 compared to DAS28<2.6 group.

DAS28, disease activity score using 28 joint count; DMD, disease modifying drug; MTX, methotrexate; TNFI, tumour necrosis factor α inhibitor.

Table 6

Linear regression analysis of variables associated with cholesterol efflux in patients with RA.

Explanatory variable	Estimate	P Value
ESR	−0.19	0.004
Prednisone use (yes/no)	2.33	0.23
Smoking (yes/no)	−0.62	0.84
Diabetes (yes/no)	1.47	0.50

ESR, erythrocyte sedimentation rate.

Table 7

Linear regression analysis of variables associated with MPO activity in patients with RA.

Explanatory	Estimate	P Value
Age (years)	0.05	0.90
Sex (male)	-0.67	0.90
HSCRП (mg/l)	1.12	0.02

HSCRП, High sensitivity C reactive protein.