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Abnormal Function of High-Density Lipoprotein (HDL) Is Associated with Poor Disease Control and an Altered HDL Protein Cargo in Rheumatoid Arthritis

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Abstract

Purpose—To characterize HDL's anti-inflammatory function in patients with rheumatoid arthritis (RA) and to identify specific differences in HDL-associated proteins and enzymes, which distinguish pro-inflammatory HDL (piHDL) from normal, anti-inflammatory HDL (aiHDL).

Methods—132 RA patients were studied. HDL's anti-inflammatory function was assessed by a cell free assay and piHDL was defined by an HDL inflammatory index (HII) ≥ 1 . Plasma and HDL-associated protein levels of apolipoprotein A-1 (apoA-1), haptoglobin (Hp), hemopexin (Hx), hemoglobin (Hb), and myeloperoxidase (MPO) were measured by direct and sandwich ELISA respectively. Lecithin:cholesterol acyltransferase (LCAT) activity was measured by a commercially available assay.

Results—Age, disease activity, presence of erosive disease, non-caucasian race, and smoking were significantly associated with piHDL in multivariate analysis. Patients with piHDL had higher measures of systemic inflammation and a significant correlation was observed between RA disease activity (DAS 28) and the HII ($r = 0.54$, $p < 0.0001$). PiHDL had higher levels of Hp, Hb, apoA-1, and MPO in HDL compared to aiHDL from RA patients (all p values < 0.05). LCAT activity was lowest in patients with piHDL, but was also significantly reduced in RA patients with aiHDL compared to healthy controls ($p = 0.001$).

Conclusions—PiHDL in this RA cohort was associated with active disease and an altered protein cargo compared to aiHDL in patients and healthy controls. HDL's anti-inflammatory function was inversely correlated with systemic inflammation in RA patients and may warrant further investigation as a mechanism by which active RA increases cardiovascular morbidity and mortality.

Premature cardiovascular disease (CVD) is a major cause of morbidity and mortality in patients with rheumatoid arthritis (RA) who have a 70% increased risk of death compared to the general population (1). Although some studies have suggested that RA mortality rates may be falling in response to new therapies (2), evidence from population-based studies of

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inception cohorts suggests that survival in RA patients has not improved, and CVD is the leading cause of death (3). Understanding mechanisms for accelerated atherosclerosis in patients with RA therefore becomes important both for appropriate treatment and aggressive primary prevention.

HDL (high density lipoprotein) cholesterol levels are inversely related to risk for atherosclerotic events (4). This protective effect of HDL arises not only from its ability to promote cholesterol efflux from artery wall cells (5), but also from anti-inflammatory properties including HDL's ability to protect LDL against oxidation (6–10). In work by Ansell and colleagues, patients with coronary heart disease (CHD) had non-protective, “pro-inflammatory HDL” (piHDL), which promoted rather than prevented accumulation of oxidized phospholipids in LDL. PiHDL induced monocyte chemotactic activity when compared to HDL from healthy controls, which was anti-inflammatory and inhibited monocyte chemotaxis (aiHDL). Patients with CHD and high serum levels of HDL (≥ 84 mg/dL) also had non-protective HDL, suggesting that HDL function may be a better predictor of atherosclerosis than standard measurements of plasma lipids (11).

Rheumatoid arthritis is a systemic auto-immune disease associated with destructive, inflammatory arthritis. Several studies suggest that systemic inflammation from active arthritis accelerates atherogenesis in RA (12;13). In work by Maradit-Kremers and colleagues with a 603 patient RA inception cohort, high clinical disease activity as measured by 3 erythrocyte sedimentation rates (ESR) of ≥ 60 mm/hour correlated with a 2-fold increased risk of death from cardiovascular disease (14).

PiHDL is increased in RA patients compared to healthy controls (15). The current study characterizes RA patients with piHDL, and shows significant associations with disease activity and damage in a cross-sectional cohort of RA patients recruited from the UCLA Rheumatology offices. Furthermore, differences in the protein composition and function of piHDL are demonstrated, and pilot work suggests that improvement in RA disease activity with methotrexate may improve HDL's anti-inflammatory capacity.

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 and sex 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 (hs-CRP) and Westergren erythrocyte sedimentation rate (ESR), and fasting lipid profiles. Cardiovascular risk and health information was obtained by questionnaire and chart review. Disease activity in RA patients was assessed by 28 tender and swollen joint counts, patient and physician global assessments on a visual analogue scale (VAS; 0–100), and patient pain, fatigue, and stiffness assessments (VAS; 0–100). A disease activity scale using the 28 joint count (DAS28) was calculated for each patient. Disease disability was assessed by the health assessment questionnaire disability index (HAQ-DI).

For the HDL function and protein 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 (16), thoroughly mixed, divided into aliquots, and kept frozen at -80°C until use.

Repeat study visits were performed in 10 RA patients who received methotrexate from their primary rheumatologist for active disease and in 10 RA patients who had no change in DMARD therapy with stable disease over the follow up period, (7.2 ± 4.3 and 7.6 ± 7.3 months, respectively, for methotrexate and stable disease groups) ($p = 0.74$). Baseline and follow-up assessments included HDL function analysis, hs-CRP, Westergren ESR, 28 tender and swollen joint counts, HAQ-DI, and patient and physician global disease assessments.

Evaluation of HDL's Anti-inflammatory Function by the Cell-Free Assay (CFA)

The CFA was a modification of a previously published method (16) using LDL as the fluorescence-inducing agent. Control LDL was prepared as described previously (16). HDL-containing supernatants were first isolated by the dextran sulfate method. 50 μl of HDL Magnetic Bead Reagent, (Polymedco catalog #5030), were mixed with 250 μl of patient plasma and incubated for 5 minutes at room temperature. The solution was then incubated for an additional 5 minutes on a magnetic particle concentrator. HDL-cholesterol in the supernatant was quantified using a standard assay (Thermo DMA Co., San Jose, CA). 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 2.0mg/ml and incubated in the dark at room temperature for 20 minutes, resulting in release of DCFH. 25 μl of LDL-cholesterol (100 $\mu\text{g}/\text{ml}$) was mixed with 50 μl of test HDL (100 μg HDL-cholesterol/ml) in black, flat bottom polystyrene microtiter plates and incubated at 37°C with rotation for 30 minutes. 25 μl of DCFH solution (0.2mg/ml) was then added to each well, mixed, and incubated at 37°C for one hour 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 cutoff of 515 nm with photomultiplier sensitivity set at medium. Readings with DCFH and LDL-C were normalized to 1.0. Readings equal or greater than 1.0 after the addition of test HDL-C indicated piHDL and values less than 1.0 indicated aiHDL. Values for intra- and interassay variability were $0.5 \pm 0.37\%$ and $3.0 \pm 1.7\%$ respectively (17).

Analysis of HDL-associated Proteins

Individual plasma samples from 16 randomly selected RA patients with piHDL, 16 RA patients with aiHDL, and 16 age and sex matched healthy controls with aiHDL were assayed for apolipoprotein A-1 (ApoA-1), hemoglobin (Hb), haptoglobin (Hp), hemopexin (Hx), and myeloperoxidase (MPO) by direct and sandwich ELISA as described below. Plasma was diluted 10-fold with PBS for all protein assays.

Proteins in plasma—ApoA-1, Hb, Hp, Hx, and MPO levels in individual plasma were quantified by direct ELISA as previously described (18). In brief, plasma samples were coated on 96-well PVC microtiter plates (BD) and incubated at 4°C overnight. Primary antibodies against human apoA-1, Hb, Hp, and Hx were used at a dilution of 1:2500 (Cambridge, MA). The primary antibodies were detected by HRP-conjugated secondary antibodies at 1:2500 dilution (Cambridge, MA). Following incubation with TMB solution (KPL, Gaithersburg, MD), HRP activity was measured at OD450. The HRP-conjugated detection antibody was used as an internal standard to convert the OD450 of each sample to the concentration of detection antibody. The value for each protein from a given sample was

calculated relative to the average concentration of that protein in healthy controls which was set to 1.

Proteins associated with HDL—The association of Hb, Hp, Hx, and MPO with HDL was determined by sandwich ELISA as described previously (18). In brief, 96-well PVC microtiter plates (BD) were precoated with 1 – 5 µg/mL of chicken anti-human HDL antibodies (Cambridge, MA) at 4°C overnight. Following incubation of the precoated plates with individual plasma, the plates were washed thoroughly and incubated with corresponding primary antibodies to human apoA-1, Hb, Hp, Hx, and MPO at 1:2500 dilution. The primary antibodies were detected by HRP-conjugated secondary antibodies at 1:2500 dilution. Following incubation with TMB solution (KPL, Gaithersburg, MD), HRP activity was measured at OD450. As above, the HRP-conjugated detection antibody was used as an internal standard to convert OD450 of each sample to the concentration of detection antibody. The value for each protein from a given sample was calculated relative to the average of that protein concentration in healthy controls, which was set to 1.

Lecithin cholesterol acyltransferase (LCAT)—The activity of LCAT was evaluated in patient and control plasma as the ratio of the intact to hydrolyzed monomer substrate (470/390 nm of emission intensity), using the test kit from Roar Biomedical (New York, NY).

Statistical analysis

Data were analyzed using JMP IN 7.0 (SAS Institute Inc., Cary, NC, USA). Patient groups were compared using Student's t-test for continuous variables and the chi-square test of association for categorical variables, along with Fisher's exact test for small sample sizes. When needed, nonparametric Wilcoxon rank-sum tests were used to analyze continuous variables. The significance level was prespecified at $p < 0.05$.

A forward stepwise logistic regression analysis was performed to evaluate correlates of piHDL in the RA cohort. Initial covariates included traditional CV risk factors (age, hypertension, LDL, HDL, diabetes, family history of CHD, smoking status, and body mass index (BMI), patient factors significantly associated with piHDL in bivariate analyses (Table 1–2), RA duration, and statin use (17). In cases where variables were highly correlated, one representative variable was chosen. An ROC curve was constructed for the model and the sensitivity, specificity, and model accuracy are reported.

Results

Demographic and Clinical Characteristics Distinguish Patients with Pro-inflammatory HDL from Patients with Anti-inflammatory HDL

RA patients with piHDL were significantly older and had higher inflammatory markers compared to patients with aiHDL (Table 1). More patients with piHDL were non-Caucasian; no significant differences in gender were noted (Table 1).

Traditional cardiovascular risk factors including standard lipoprotein levels were similar between RA patients with piHDL and aiHDL, however there were more current smokers and patients with diabetes in the piHDL group ($p < 0.05$) (Table 1). Measures of RA disease activity and severity were significantly increased in patients with piHDL compared to aiHDL including DAS28, tender/swollen joint counts, patient/physician global disease assessments, erosive disease, and disability measured by the health assessment questionnaire disability index (HAQ-DI) (Table 2). Significant positive correlations were also seen between the HDL inflammatory index (HII) and DAS28, Westergren ESR, and HS-CRP

(Figure 1). Worse HDL anti-inflammatory function was associated with higher DAS28 scores and levels of systemic inflammation (Figure 1). Patients with piHDL were less likely to be taking methotrexate compared to patients with anti-inflammatory HDL ($p = 0.01$). No other differences in DMARD or COX-1/COX-2 inhibitor use were seen between patients with piHDL and aiHDL (Table 2).

Correlates of Pro-inflammatory HDL in Patients with Rheumatoid Arthritis

Multivariate logistic regression analysis was performed to determine correlates of piHDL in the RA cohort. Age, disease activity, and the presence of erosive disease were significant positive correlates of piHDL ($p < 0.05$) after controlling for traditional and RA related CV risk factors (Table 3). Smoking at the time of the study was also a significant correlate of piHDL while caucasian race and methotrexate use were protective (Table 3). The accuracy of the model was 85% with a sensitivity of 81% and a specificity of 88%.

Composition and Function of Pro-inflammatory HDL is Different from Anti-inflammatory HDL in Patients with RA

Patients with piHDL had significantly higher levels of Hp, Hb, apoA-1, and MPO associated with HDL compared to patients with aiHDL (p values < 0.05 ; MPO, ($p = 0.05$)) (Figure 2). Hx levels were higher in plasma of patients with piHDL, ($p = 0.0003$), but were not elevated in HDL (Figure 2). Significant correlations ($p < 0.05$) of HDL's anti-inflammatory function were observed with HDL-associated MPO ($r = 0.43$), apoA-1 ($r = 0.42$), Hb ($r = 0.48$), and Hp ($r = 0.67$) in patients with RA (all p values < 0.05).

LCAT activity was lowest in patients with piHDL, but was also significantly reduced in RA patients with aiHDL compared to age and sex matched healthy controls ($p = 0.001$). A modest but significant correlation was found between plasma Hp and LCAT activity ($r = -0.30$; $p = 0.04$). Higher Hp levels were associated with lower LCAT activity. There was also a trend for correlation of HDL-associated Hp with LCAT activity ($r = -0.26$; $p = 0.08$). No other HDL-associated proteins studied were correlated with LCAT activity except for Hb for which plasma but not HDL-associated levels were modestly correlated ($r = -0.29$; $p = 0.04$ and $r = -0.09$; $p = 0.53$ respectively).

Methotrexate Improves HDL's Anti-inflammatory Function in RA Patients

10 RA patients with active disease who were started on methotrexate and had significant improvements in disease activity (DAS 28), disease disability (HAQ-DI), and inflammatory markers following treatment (p values < 0.05), were compared with 10 patients with no changes in DMARD therapy and with stable disease (Table 4). The HDL inflammatory index (HII) was significantly improved in the active disease group treated with methotrexate ($p = 0.009$), however was not significantly changed in the stable disease group ($p = 0.62$). The change in HII in the methotrexate group was also significantly greater compared to the change in HII in the stable disease group ($p = 0.002$). For the 20 patients, regardless of therapy, a strong correlation was found between the change in disease activity, ($\% \Delta$ DAS 28), and the change in HII, ($\% \Delta$ HII), ($r = 0.73$; $p = 0.0002$). This correlation was also seen in limited analysis of the 10 patients receiving methotrexate ($r = 0.68$, $p = 0.0289$).

Discussion

The mortality gap between RA patients and the general population has been growing for several decades, largely because of excess cardiovascular deaths in patients with RA (3). While research regarding this increased cardiovascular morbidity and mortality in RA has burgeoned in recent years, a need for biomarkers to evaluate the effects of disease modifying therapies and to identify high risk patients for prevention strategies remains. Traditional

CHD risk factors and Framingham risk guidelines alone are not sufficient to identify RA patients at high risk for CHD.

Substantial evidence has accumulated that HDL which has become “dysfunctional” may play an important role in the development of atherosclerosis (11). Detection of piHDL has also been identified as a potentially useful marker for gauging susceptibility to atherosclerosis (7;11;16;19–21) and piHDL is increased in patients with systemic lupus erythematosus and RA (15). The current work characterizes RA patients with piHDL in a cross-sectional cohort of RA patients from the UCLA Rheumatology offices and identifies specific differences in the protein structure and function of piHDL, which may account in part for its abnormal protective capacity.

Systemic markers of inflammation such as hs-CRP predict coronary events in patients with or without CVD in the general population (22–24). Similarly, several studies suggest that systemic inflammation accelerates atherogenesis in RA, perhaps by accentuation of both established as well as novel risk factor pathways (1;12;14;25). The current work demonstrates a significant association between systemic inflammation and HDL function in the RA cohort studied. Higher levels of systemic inflammation were associated with worse HDL protective capacity. The HDL inflammatory index was strongly correlated with disease activity measured by the DAS28, and patients with abnormal, piHDL had higher sedimentation rates and hs-CRP compared to patients with aiHDL. Measures of disease severity including the presence of erosive disease and disability as measured by the HAQ-DI, a strong predictor of mortality in epidemiologic studies (26), were also associated with piHDL. While these associations do not prove causality, they do suggest that abnormal HDL function may warrant further investigation as a potential mechanism for the increased CV risk in patients with RA. The major limitation of the current study is that the sample size of 132 patients is small, selective, and highly susceptible to both volunteer and referral bias.

Work by our group has previously shown that HDL function in RA patients may be modestly improved with high dose statin therapy (17). The current pilot work suggests that improvement in active disease with methotrexate may have beneficial effects on HDL’s function. Given the potential for a biased analysis due to a small and highly selected sample, these results must be considered hypothesis generating. Large, prospective controlled studies are necessary to confirm these results as well as to determine if methotrexate and other DMARDs have intrinsic beneficial effects on HDL function, or if improvement in HDL’s protective capacity is largely secondary to improvement in disease activity. Popa and colleagues have recently shown that treatment with infliximab for 6 months improves HDL’s anti-oxidant function (27). Epidemiologic work has also previously suggested that methotrexate and anti-TNF- α therapies may have beneficial effects on CV mortality in RA (28–30).

In addition to chronic inflammatory processes, several investigators have suggested that traditional CV risk factors may contribute to the increased coronary morbidity and mortality seen in RA (25;31). Age is a known coronary risk factor in the general population including RA patients, and was significantly correlated with piHDL in the multivariate analysis of our RA cohort. Work by Wick and colleagues has previously described an association of increasing age with abnormalities in lipoprotein metabolism, including decreased fluidity of monocyte plasma membranes, and decreased HDL-mediated cholesterol efflux in older patients (32). We hypothesize that older RA patients may not effectively compensate for the pro-inflammatory oxidized phospholipids which accumulate in HDL during chronic inflammation and inhibit HDL-associated antioxidant enzymes (33). The activity of LCAT, an HDL-associated enzyme involved in reverse cholesterol transport, showed a modest but significant correlation with age in our patients ($r = -0.30$, $p = 0.036$). Current smoking was

also a correlate of piHDL in multivariate analysis, suggesting that other traditional CV risk factors may influence HDL's protective function as previously reported (34).

We next investigated several HDL-associated proteins linked to abnormal HDL function or active inflammation and identified specific differences in HDL-associated proteins and enzymes, which distinguished piHDL from aiHDL in our RA cohort. Watanabe and colleagues previously reported an association of hemoglobin (Hb) with HDL, demonstrating that Hb was differentially associated with piHDL in both CHD patients and hyperlipidemic, atherosclerotic mice. HDL containing Hb consumed nitric oxide and contracted arterial vessels *ex vivo* (18). RA patients with piHDL from our cohort also had significantly higher levels of hemoglobin associated with HDL, compared to both RA patients with aiHDL and healthy controls.

Myeloperoxidase (MPO) is an enzyme which utilizes hydrogen peroxide (H₂O₂) and a variety of low-molecular weight organic and inorganic substances as substrates to form reactive oxidant species (35;36). Measurement of plasma MPO levels independently predicts early risk of MI in the general population (37), and increased MPO activity has recently been described in RA patients (38). MPO was increased in HDL of RA patients with piHDL compared to patients with aiHDL in our study. We hypothesize that MPO may oxidatively alter protective proteins in HDL such as apoA-1, thereby inhibiting HDL's normal anti-inflammatory capacity.

Because RA is a systemic inflammatory disease, we next evaluated two acute phase proteins, haptoglobin and hemopexin, which bind hemoglobin and heme respectively, and have been identified in association with HDL (39;40). Both proteins were significantly elevated in plasma of RA patients with piHDL compared to aiHDL, however, only haptoglobin, was significantly elevated in HDL of RA patients with piHDL compared to patients with aiHDL. HDL-associated haptoglobin also had the strongest correlation with HDL function of the HDL-associated proteins studied. Higher haptoglobin in HDL was associated with worse HDL anti-inflammatory function.

Finally, while interesting, this data must be considered hypothesis generating as it is limited by both sample size and selection bias. Future work will pursue additional HDL-associated protein analysis in larger RA cohorts in order to better understand how HDL is altered in the setting of active RA, as well as to consider whether a panel of HDL-associated proteins can serve as a surrogate marker for the current HDL function assay.

In summary, RA patients in our cohort with abnormal HDL function were more likely to have active, erosive disease. Traditional CV risk factors such as smoking may also contribute to poor HDL protective capacity. PiHDL was associated with alterations in the protein cargo of HDL, which may adversely affect its functional capacity. The current work proposes a mechanism for CV risk in RA whereby ongoing disease activity leads to changes in the structure and function of HDL, impairing its ability to prevent LDL oxidation and the development of atherosclerosis. Further large, prospective studies are necessary to determine whether HDL function is a biomarker of CV risk in RA. Drugs such as apoA-1 mimetic peptides, which improve HDL function and reduce atherosclerosis in animal models, are currently in clinical trials of patients with CHD (41). These agents have shown modest disease modifying effects in an animal model of RA(42) and may have particular benefits for patients with both inflammatory arthritis and increased CV risk.

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Figure 1.
Correlation of Inflammatory Markers and DAS28 with HDL Inflammatory Index (HII).

Table 1

Demographic and Clinical Data of RA Patients with Pro-inflammatory HDL Compared to RA Patients with Anti-inflammatory HDL *

	Patients with Anti-inflammatory HDL (n=102)	Patients with Pro-inflammatory HDL (n=30)	P value
HDL Inflammatory Index (HII) CFA	0.42 ± 0.23	1.29 ± 0.25	<0.0001
Age, years	53 ± 15	60 ± 11	0.006
Female, %	88.2	83.3	0.33
Caucasian, %	56.9	33.3	0.037
ESR, mm/h	23 ± 20	57 ± 31	<0.0001
HSCRP, mg/l	5.8 ± 10.1	31.0 ± 43.3	<0.0001
Body Mass Index, lb/in2	26.5 ± 6.2	27.6 ± 7.2	0.47
Hypertension, %	22.8	36.7	0.16
Diabetes, %	5.9	26.7	0.004
Current Smoker, %	4.1	17.2	0.03
Family History, %	6.6	17.9	0.13
Total Cholesterol, mg/dL	189 ± 37	189 ± 43	0.99
LDL Cholesterol, mg/dL	106 ± 32	104 ± 37	0.73
HDL Cholesterol, mg/dL	58 ± 16	54 ± 21	0.12
Triglycerides, mg/dL	123 ± 65	148 ± 87	0.16
Exercise, %	57	48	0.52

* Except where indicated otherwise, values are the mean ± SD.

Table 2

Disease Characteristics and Medication Use in RA Patients with Pro-inflammatory HDL Compared to RA Patients with Anti-inflammatory HDL *

	Patients with Anti-inflammatory HDL (n=102)	Patients with Pro-inflammatory HDL (n=30)	P value
RA Duration, years	11.5 ± 11.7	12.0 ± 11.6	0.95
28 Tender Joints	8 ± 9	17 ± 9	<0.0001
28 Swollen Joints	5 ± 5	11 ± 7	<0.0001
DAS28	4.3 ± 1.8	6.2 ± 1.9	0.001
Patient Pain †	35 ± 29	67 ± 30	<0.0001
Patient Global †	39 ± 29	67 ± 31	<0.0001
Physician Global †	33 ± 24	61 ± 30	<0.0001
Patient Stiffness †	33 ± 28	60 ± 35	0.0005
Patient Fatigue †	45 ± 28	62 ± 30	0.007
HAQ-DI	0.74 ± 0.75	1.58 ± 0.89	<0.0001
RF Positive, %	77	86	0.44
Erosive Disease, %	66.3	92.6	0.007
Statin, %	15.7	26.7	0.18
COX-1 inhibitor, %	32.7	48.3	0.13
COX-2 inhibitor, %	8.9	6.7	1.0
Prednisone, %	24.8	26.7	0.81
Methotrexate, %	62.8	36.7	0.01
Leflunomide, %	8.9	6.7	1.0
Sulfasalazine, %	5.9	0	0.33
Hydroxychloroquine, %	16.8	13.3	0.78
TNF-α inhibitor, %	41.6	30.0	0.29

* Except where indicated otherwise, values are the mean ± SD.

† Per 100 mm Visual Analogue Scale.

Table 3

Stepwise Logistic Regression Analysis of Variables Associated with Pro-Inflammatory HDL in Patients with RA

Explanatory Variable	*Odds Ratio	95% CI	P Value
Age, years	1.127	1.052–1.226	0.0003
Das28	1.987	1.315–3.269	0.0006
Erosive Disease	21.23	2.450–375.8	0.0032
Methotrexate Use	0.224	0.043–0.986	0.0478
Caucasian	0.107	0.015–0.576	0.0081
Current Smoker	10.58	1.355–127.03	0.0234
HDL, mg/dL	1.032	0.994–1.077	0.1040

* Odds ratios for continuous variables age, das28, and HDL reflect one unit change in the predictor—i.e. per one year in age, 1.0 unit change in das28, and 1.0 mg/dL in HDL.

Table 4

Changes in Disease Markers and the HDL Inflammatory Index in 10 Patients After Starting Methotrexate Compared to 10 Patients with Stable Disease and no Change in DMARD Therapy*

	Follow-up (months)	Δ HII	Δ AS28	Δ HQAQ-DI	Δ ESR (mm/h)	Δ HS-CRP (mg/L)
Methotrexate	7.2 $\pm$ 4.3	-0.46 $\pm$ 0.3	-3.9 $\pm$ 1.4	-1.1 $\pm$ 1.0	-43 $\pm$ 32	-8.3 $\pm$ 10.1
Stable Disease	7.6 $\pm$ 7.3	0.004 $\pm$ 0.16	-0.4 $\pm$ 0.7	-0.01 $\pm$ 0.3	-8 $\pm$ 16	1.9 $\pm$ 3.8
p value	0.74	0.002	0.0003	0.02	0.005	0.02

* Except where indicated otherwise, values are the mean $\pm$ SD.