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Associations between disease activity, markers of HDL functionality and arterial stiffness in patients with rheumatoid arthritis

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ABSTRACT

Background and aims: Rheumatoid arthritis (RA) is a chronic, inflammatory disease associated with increased risk of cardiovascular disease (CVD). Measures of HDL metabolism/function were shown to be altered in RA patients with high disease activity. We aimed at evaluating the effect of HDL characteristics on arterial stiffness in RA patients classified according to the inflammatory disease activity.
Methods: RA patients were classified according to disease activity (DAS-28) into active RA (n = 27; DAS-28 > 3.2) and inactive RA patients (n = 17; DAS-28 < 3.2). A control group of healthy individuals was also studied (n = 33). Clinical and biochemical characteristics, cholesteryl ester transfer protein (CETP) and paraoxonase 1 (phenylacetate and paraoxonase) activities and carotid-femoral pulse wave velocity (cf-PWV) were determined.
Results: Anthropometric characteristics were similar in all groups. In accordance with the inflammatory status, active RA patients presented elevated hsCRP levels ($p < 0.001$). There were no differences in the lipid profile between groups. Similarly, features of insulin resistance were absent in RA patients (p = non-significant). Active RA patients presented higher CETP activity than the other two groups ($p = 0.026$). Phenylacetate and paraoxonase activities were altered in active RA patients in comparison with the other groups ($p = 0.034$ and $p = 0.041$, respectively). Cf-PWV was significantly higher in active RA patients in comparison with controls, following adjustment by age ($p = 0.030$). Age ($\beta_{st} = 0.468$, $p = 0.013$) and apo A-I levels ($\beta_{st} = -0.405$, $p = 0.029$) were independent predictors of cf-PWV in a model including hsCRP, HOMA-IR, and phenylacetate activity ($r^2 = 0.42$).
Conclusions: High DAS-28 identifies patients with alterations in HDL characteristics. Plasma levels of apo A-I can be used as a marker of arterial stiffness in RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease associated with increased mortality and morbidity, predominantly as a result of cardiovascular disease (CVD) [1]. An approximately 2-fold increase in mortality from myocardial infarction and stroke has been observed in studies comparing RA patients with the general population [1–3]. Such increased risk of CVD cannot be completely explained by traditional risk factors [4], and, therefore, cannot be fully predicted by the conventional CVD risk markers.

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Dyslipidemia is not frequent in RA patients [5,6] and, if present, the most common lipid alteration involves low levels of high-density lipoprotein-cholesterol (HDL-C) concentration. Nonetheless, despite the presence or absence of low HDL-C, HDL functionality can be altered in RA patients. Recently, it was shown that HDL cholesterol efflux capacity was associated with subclinical atherosclerosis [7] and incident cardiovascular events independently of HDL-C levels [8]. Therefore, metrics of HDL functionality can be more informative than lipoprotein levels. The main limitation is that HDL functionality assays are laborious and hard to standardize. Up to the moment, none of the available assays are suitable for their use in the general practice. In its place, surrogate metrics of HDL functionality, such as the concentration of apolipoprotein (apo) A-I [9], the major functional and structural component of HDL, and the activity of paraoxonase 1 (PON 1) can be relevant. Such analysis and its impact on measurements of vascular compliance and inflammation have been scarcely studied in RA.

PON 1 is an antioxidant enzyme exclusively associated with HDL which plays a major function in preventing lipid oxidation [10]. PON 1 is important for the protection of endothelial cells from damage derived from oxidized phospholipids [10]. RA patients presented lower PON 1 activity than controls even in the absence of changes in the lipid profile [11,12]. Such decrease was associated with inflammation and was partially reversed by treatment with biological agents [13].

Cholesteryl ester transfer protein (CETP) is a protein which mediates the transfer of neutral lipids (cholesteryl esters and triglycerides) between HDL and apo B-containing lipoproteins. Therefore, CETP is one of the determinants of HDL chemical composition and HDL plasma subfraction distribution, which, in turn, influence HDL functionality [14]. Therefore, elevated CETP activity can be a factor associated with HDL dysfunction in RA, as it has been demonstrated in patients with metabolic syndrome and type 2 diabetes [15,16].

Carotid to femoral pulse wave velocity (cf-PWV) is a measure of vascular compliance which correlates with active inflammation and has been associated with increased CVD risk in RA [17,18]. Accordingly, cf-PWV was increased in RA patients *vs.* controls [17,18], and this alteration was modified by treatment with biological agents [19,20]. However, the relationship between metrics of HDL functionality and cf-PWV has not been assessed yet. The aim of the present study was to evaluate the effect of HDL characteristics on arterial stiffness in RA patients classified according to the disease inflammatory activity.

2. Materials and methods

2.1. Subjects

Forty four patients with clinical manifestations of RA were recruited at José de San Martín Clinical Hospital, University of Buenos Aires, and at Buenos Aires Italian Hospital (Buenos Aires, Argentina) from April 2009 to January 2010. All patients met the 1987 revised RA criteria of the American College of Rheumatology [21]. RA patients were classified according to the disease activity score using 28 joint count and the erythrocyte sedimentation rate (DAS-28) [22]. Moderate-high disease activity was defined as a DAS-28 score >3.2 (Active RA, n = 27) and low disease activity as a DAS-28 score <3.2 (Inactive RA, n = 17).

Among the group of active RA patients, 10 patients were on methotrexate (MTX) monotherapy and 14 were on DMARDs combination therapy. Six patients were receiving MTX + hydroxychloroquine (HCQ), 6 were on MTX + tumor necrosis factor (TNF) inhibitor therapy, one was taking MTX + a pyrimidine synthesis inhibitor and one was on MTX + rituximab + HCQ. Finally, one patient was taking a pyrimidine synthesis inhibitor as monotherapy, one TNF inhibitor therapy as monotherapy and one patient was treatment naïve. Among the group of inactive RA patients, 9 of them were on MTX monotherapy and 6 were on DMARD combination. Four patients were on MTX + HCQ and 2 were taking a pyrimidine synthesis inhibitor + HCQ. Of the rest of the patients, one was on TNF inhibitor therapy as a monotherapy and one was treatment-naïve. Importantly, there were no significant differences in the treatment modalities between the groups (Supplementary Table 1). In addition, there were no differences in the consumption of nonsteroidal anti-inflammatory drugs (NSAIDs) between active and inactive RA patients (15/27 *vs.* 6/17; $p = 0.124$). Biological agents were administered to 8/27 and 1/17 of active and inactive RA patients, respectively ($p = 0.121$), mostly as an add-on therapy to MTX. Corticoids were only taken by active RA patients (8/27 *vs.* 0/17, $p = 0.015$). Patients were not advised to suspend medication prior to the study to accurately evaluate the balance between atherogenic and antiatherogenic factors. No medication changes were registered in a two-week period prior to the blood extraction.

Exclusion criteria for the patient selection were: presence of infectious diseases, diabetes or any previous cardiovascular event, treatment with drugs capable of producing vasculitis or cardiac dysfunction and use of antioxidants and/or lipid-lowering drugs during the last month. Thirty three healthy, normolipidemic, middle-aged subjects were recruited to form a control group. Written informed consent was obtained from all participants and the study was approved by the Ethics Committees from School of Pharmacy and Biochemistry, University of Buenos Aires, and from the Buenos Aires Italian Hospital in accordance with local institutional guidelines conformed to the Declaration of Helsinki.

2.2. Blood samples

Blood samples were withdrawn from the antecubital vein of each participant at the time of recruitment after 12 h overnight fast. Serum and EDTA plasma (final EDTA concentration: 1 mg/ml) were prepared from venous blood collected into sterile, evacuated tubes (BD, Vacutainer®). Plasma was immediately separated by low-speed centrifugation at 4 °C; serum and plasma were aliquoted and frozen at −80 °C under nitrogen; each aliquot was thawed only once directly before analyses.

2.3. Clinical and biological parameters

Complete blood count was determined in a Coulter® GEN-S autoanalyser (Beckman Coulter, Fullerton, CA, USA.). Erythrocyte sedimentation rate (ESR) was determined using Test 1 analyser (Alifax, Padova, Italy). Plasma levels of total cholesterol (TC), triglycerides (TG), glucose, urea, uric acid, creatinine, albumin, aspartate amine transferase (AST), alanine amine transferase (ALT) and alkaline phosphatase (ALP), were measured by standardized methods in a COBAS® C501 autoanalyser (Roche, Mannheim, Germany). LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) were determined by selective precipitation methods. Plasma apo A-I, apo B and high-sensitivity C-reactive protein (hsCRP) were quantitated by immunoturbidimetry (Roche, Mannheim, Germany). Serum amyloid A (SAA) and rheumatoid factor (RF) were determined by nephelometry (Siemens, Munich, Germany) and insulin levels by radioimmunoassay (DPC, Los Angeles, California, USA). Antibodies anti-cyclic citrullinated peptides (Anti-CCP) were measured by a 2nd generation immunoassay (INOVA Diagnostics, San Diego, CA, USA).

2.4. Activities of lipoprotein-associated proteins and enzymes

2.4.1. Cholesteryl ester transfer protein activity

Cholesteryl ester transfer protein (CETP) activity was determined in serum samples according to the general procedure previously described by Lagrost et al. [23]. Results were expressed as nmol of ^{3}H-cholesteryl esters transferred from HDL3 to apo B-containing lipoproteins, per ml, per hour. All measurements were carried out within the same assay. Within-run precision was 4.9%.

2.4.2. Paraoxonase and arylesterase activities

Activity of PON 1 was evaluated employing two different substrates, paraoxon and phenylacetate (Sigma Chemical Co, St. Louis, MO, USA.; PON and ARE activities, respectively). Both activities were measured in serum samples following the method of Furlong et al. [24]. Results were expressed as $nmol.ml^{-1}.min^{-1}$ and $\mu mol.ml^{-1}.min^{-1}$ for PON and ARE activities, respectively. All Measurements were carried out within the same assay. Within-run precision for PON and ARE activities were 4.6% and 4.2%, respectively. PON phenotypes were determined by the double substrate method [25].

2.5. Pulse wave velocity

Carotid-femoral pulse wave velocity was measured in a subset of 12 inactive RA and 10 active RA patients, as well as in 5 controls, who were randomly selected from the whole study population to reflect its major clinical and biochemical characteristics. An automatic non-invasive device was used (Complior®; Colson AS, París, France). Briefly, this method measures the pulse wave at two points of the vessel separated by a previously measured distance, automatically determining the translation speed of the wave between the two points. Both the validation and reproducibility of this method were previously reported [26,27].

2.6. Statistical analysis

Power analysis was performed based on studies found in the literature regarding mostly PON 1 activity, as well as other HDL markers analyzed [12,28,29]. In the cited studies, an effect size of 0.8 standard deviations was commonly observed. Then, having set a power of 0.8, a significance level (α) of 0.05 in a two tail test and an allocation relationship of 2: 1 (patients: controls), the minimum number of subjects to be included was 40 for the RA patients and 20 for the control group.

Distributions of all variables were analyzed for normality using the Shapiro-Wilks test. Normally distributed variables are expressed as mean ± SD; non-Gaussian distributed variables are expressed as median (interquartile range). Between-group differences in normally-distributed variables were analyzed using ANCOVA test employing age as covariate and Tukey *post-hoc* test. For non-Gaussian distributed variables, log-transformation and ANCOVA test was used. Variables that did not follow normal distribution after mathematical transformations were compared using the Kruskall-Wallis test. Differences between the groups of active RA patients and healthy controls were evaluated by linear contrasts in the ANCOVA test. Differences in dichotomous variables were analyzed by Chi-square test applying Bonferroni correction for multiple comparisons. Spearman's correlation coefficients were calculated to evaluate relationships between variables. Multiple linear regression was performed to assess the variables associated with cf-PWV. The predictors in the model were age, hsCRP, HOMA-IR, Apo A-I and ARE activity. Two tailed p values <0.05 were considered significant. The statistical software INFOSTAT® (Grupo INFOSTAT, National University of Córdoba, Córdoba, Argentina) and SPSS® 17.0 (Chicago, Ill, USA) were used for all data and statistical analyses.

3. Results

3.1. Clinical, biochemical and metabolic characteristics

Mean DAS-28 score was significantly higher in active RA patients compared with inactive RA patients (4.2 ± 0.9 *vs.* 2.5 ± 0.4, $p < 0.001$). Median disease duration was 9 years (interquartile range: 6–12 years). There were no differences in disease duration between active and inactive RA patients (9 (4–16) *vs.* 9 (6–16) years, $p = 0.860$, respectively). Anti-CCP antibodies were positive in 86% of the patients in whom they were measured (n = 21). RF was positive in 79% of patients in whom data was available (n = 20). There were no differences in the number of positive patients for autoantibodies between active and inactive RA groups (RF positive: 7/12 *vs.* 8/9, $p = 0.125$, for active and inactive RA patients, respectively. Anti-CCP positive: 11/12 *vs.* 7/8, $p = 0.761$, for active and inactive RA patients, respectively).

Clinical and biochemical characteristics of patients and controls are shown in Table 1. Sex distribution, BMI, waist circumference and number of smokers were similar in all the groups studied. RA patients were significantly older than the controls. In consequence, all the differences among the groups were analyzed following adjustment for age. In accordance with the inflammatory status, active RA patients presented elevated ESR and hsCRP levels and reduced albumin concentration relative to the other groups. However, no differences were observed between the groups in plasma SAA levels. Besides, ALT activity was higher in active relative to inactive RA patients (Table 1).

Metabolic characteristics of RA patients and healthy controls are shown in Table 2. Plasma levels of markers of insulin resistance, as well as the lipoprotein profile, were similar between the groups. However, when employing ANCOVA with linear contrasts, significantly lower HDL-C levels were found in active RA patients following adjustment by age ($p = 0.047$).

Active RA patients presented higher CETP activity than the other two groups (Fig. 1). CETP activity was positively correlated with waist circumference (r = 0.38, $p = 0.003$), triglycerides (r = 0.34, $p = 0.003$), HOMA-IR (r = 0.24, $p = 0.050$) and hsCRP (r = 0.29, $p = 0.014$), while negatively with apo A-I concentration (r = −0.24, $p = 0.050$).

3.2. Markers of antioxidative capacity of HDL

PON 1 activity was evaluated by its arylesterase (ARE) and paraoxonase (PON) activities. ARE activity was significantly reduced in patients with active RA in comparison to controls (Fig. 2). No significant differences between the groups were observed in the distribution of PON 1 phenotypes (AA: 35, 30, 30; AB: 59, 67, 60; BB: 6, 3, 10%, for active, inactive RA and control subjects, respectively; $p = 0.571$). Therefore, PON activity was compared across the groups without phenotype adjustments. A significant trend towards reduced PON activity was noticed in active RA (Fig. 2).

3.3. Arterial stiffness

It is important to note that the randomly selected subgroup of patients and controls that underwent cf-PWV assessment was not different from the general study population (data not shown). Cf-PWV was compared using ANCOVA with linear contrasts and significantly higher levels were observed in RA patients in comparison with the controls following adjustment by age (Fig. 3). Such

result indicates that a high disease activity might be needed to significantly affect arterial stiffness. Cf-PWV correlated with age ($r = 0.68$, $p < 0.001$), hsCRP ($r = 0.50$, $p = 0.009$), HOMA-IR ($r = 0.41$, $p = 0.042$) and apo A-I levels ($r = -0.42$, $p = 0.028$). To assess the relationship between metrics of HDL antioxidative capacity with arterial stiffness, a multiple linear regression analysis including age, hsCRP, HOMA-IR, apo A-I and ARE activity as independent variables was carried out (Table 3). Age and apo A-I were found to independently predict 42% of the variance in cf-PWV.

4. Discussion

The present study explores the associations between cf-PWV, as a marker of arterial stiffness, and metrics of HDL function in RA patients. Cf-PWV was higher as RA patients presented higher disease activity score. In addition, a gradual reduction in HDL-C and PON 1 activity, and an increase in CETP activity were also observed. In contrast, no features of insulin resistance were evidenced in RA patients. Therefore, inflammation and high disease activity are accompanied by the presence of altered metrics of HDL function and arterial stiffness in RA.

PWV increases in RA patients as a consequence of structural and functional alterations caused by inflammation [20]. Therefore, cf-PWV constitutes one of the preferred markers of vascular dysfunction in RA patients [30]. Cf-PWV was significantly correlated with age, hsCRP, HOMA-IR and apo A-I levels. Age, hsCRP and DAS-28 have been previously identified as independent predictors of arterial stiffness in RA patients [17,31]. However, in the present study, a crucial role was also pointed out for plasma levels of apo A-I. Such association may reflect the protective role of HDL for vascular health. Indeed, plasma HDL-C concentrations were not correlated with cf-PWV (data not shown), highlighting the reading of apo A-I concentrations as a marker of HDL functionality.

The relatively low mean difference in cf-PWV (MD = 0.9 m/s) between active RA patients and the group of controls might be a consequence of the advanced stage of RA (median disease duration: 9 years, IQR: 4–16 years). Ambrosino et al. [17] in their meta-analysis identified a similar mean difference (MD = 1.32 m/s) for late RA patients *vs.* controls. The slightly lower mean difference in cf-PWV observed in the present study might be related to the relatively low number of patients who underwent cf-PWV measurement. However, in accordance to the sign and significance of the correlations observed, the differences would probably be more pronounced if more active RA patients (characterized by high levels of inflammatory markers) or controls were studied. The present results are in contrast with those published recently by Arida et al. [32]. In their study, cf-PWV was not significantly elevated in RA patients with no signs of traditional cardiovascular risk factors *vs.* controls. The difference between these studies, both conducted in RA patients who were intensively treated with DMARDs and did not present evident signs of dyslipidemia, may be mainly attributed to two facts: 1) the series of patients from the study of Arida et al. [32] showed a higher use of biological agents (41%) which were shown to significantly reduce arterial stiffness as compared to our study (20%) and 2) the groups of active RA and inactive RA patients were not compared by separate to the controls. This last issue results of crucial importance as DAS-28 and other inflammatory markers were shown to be intimately associated with arterial stiffness [17,18].

Apo A-I is the main protein component of HDL and it is involved in various antiatherogenic actions such as cell cholesterol efflux and antioxidative functions [33–35]. In addition, apo A-I has been shown to protect endothelial cells from inflammation and to facilitate vascular relaxation through its effects on nitric oxide (NO) signaling [36,37]. In this context, the finding of a significant association between apo A-I levels and cf-PWV can be a consequence of increased vascular damage in RA patients with decreased apo A-I concentration. In fact, a recent study showed that anti-inflammatory treatment was associated with an increase in HDL cholesterol efflux capacity and apo A-I levels in RA patients [38].

There is a growing body of evidence that both acute and chronic inflammatory conditions induce compositional and functional modifications of apo A-I and HDL [39,40]. In our study, PON 1 activity (measured either as arylesterase or paraoxonase activity) was used as a surrogate marker of HDL antioxidative capacity similarly to other reports [41,42]. Although PON 1 role in HDL antioxidative activity has been questioned [43], its correlation with HDL functional characteristics is well supported [44]. Therefore, the lower PON 1 activity observed in the active RA patients may indicate the presence of functionally deficient HDL particles. Another common

Table 1
Clinical and biochemical characteristics of RA patients, grouped according to disease activity score, and of healthy controls.

	Active RA patients (n = 27)	Inactive RA patients (n = 17)	Healthy controls (n = 33)	*p*
Males/females (n)	2/25	3/14	7/26	0.330
Age (years)	56 ± 9[a]	56 ± 12[a]	49 ± 11[b]	0.030
BMI (kg/m^2)	27.7 (24.7–30.6)	24.8 (19.4–26.8)	24.3 (22.8–27.3)	0.080
Waist (cm)	97 (83–106)	81 (74–100)	85 (77–93)	0.320
ESR (mm)	27 (23–37)[a]	17 (13–22)[b]	15 (10–22)[b]	<0.001
Tobacco use (n)	10	7	12	0.946
Hypertension (n)	4	2	–	0.832
hsCRP (mg/l)	5.4 (3.2–10.2)[a]	1.3 (0.5–4.1)[b]	1.2 (0.8–1.6)[b]	<0.001
SAA (mg/l)	7.6 (4.9–13.6)	6.9 (3.0–10.5)	6.0 (3.5–10.6)	0.538
Hb (g/l)	1.28 ± 0.09	1.34 ± 0.13	1.33 ± 0.14	0.215
WBC (10^3 cells/ml)	7.1 (5.7–8.1)	6.8 (4.8–7.6)	6.4 (5.5–7.0)	0.673
Urea (mmol/l)	4.7 (4.2–5.5)	5.3 (4.0–6.3)	5.3 (4.0–5.8)	0.415
Creatinine (μmol/l)	61.9 (51.3–68.1)	58.3 (53.9–75.1)	63.6 (53.9–70.7)	0.218
Uric acid (μmol/l)	243 (208–285)	220 (155–256)	220 (196–327)	0.325
Albumin (g/l)	0.38 ± 0.04[a]	0.41 ± 0.03[b]	0.42 ± 0.04[b]	0.003
Bilirubin (μmol/l)	10.3 (8.6–12.0)	12.0 (10.3–13.7)	8.6 (6.8–12.0)	0.641
AST (IU/l)	25(21–28)	23(20–24)	20(19–25)	0.087
ALT (IU/l)	21(16–27)[a]	16(10–20)[b]	15(13–21)[ab]	0.049
ALP (IU/l)	72(64–94)	68(55–85)	57(50–81)	0.060

RA, reumathoid arthritis; BMI, body mass index; Ht, hematocrit; Hb, hemoglobin; WBC, white blood cells; ESR, erythrocyte sedimentation rate; hsCRP, high sensitivity C reactive protein; SAA, serum amyloid A; AST, aspartate-amine transferase; ALT, alanine-amine transferase; ALP, alkaline phosphatase.
Data are shown as mean ± SD or median (interquartile range) according to data distribution. Differences were evaluated by ANCOVA using age as covariate. Different letters depict groups with significant differences according to Tukey's *post-hoc* test.

Table 2
Metrics of glucose and lipoprotein metabolism in RA patients, grouped according to disease activity score, and in healthy controls.

	Active RA patients (n = 27)	Inactive RA patients (n = 17)	Healthy controls (n = 33)	*p*
Glucose (mmol/l)	5.2 ± 0.6	5.1 ± 0.7	4.9 ± 0.5	0.512
Insulin (mU/l)	7.8 (5.1–10.3)	7.3 (4.8–9.1)	6.9 (4.5–10.3)	0.253
HOMA-IR	1.8 (1.3–2.2)	1.8 (1.1–2.2)	1.6 (0.9–2.4)	0.875
TG (mmol/l)	1.08 (0.73–1.46)	0.89 (0.70–1.07)	0.83 (0.63–1.11)	0.228
TC (mmol/l)	5.1 ± 0.9	5.2 ± 0.9	5.4 ± 0.8	0.231
HDL-C (mmol/l)	1.37 (1.16–1.68)	1.45 (1.24–1.76)	1.65 (1.27–1.94)	0.130
LDL-C (mmol/l)	3.4 ± 0.6	3.5 ± 1.0	3.7 ± 0.9	0.531
Apo A-I (g/l)	1.42 ± 0.25	1.40 ± 0.17	1.49 ± 0.22	0.126
Apo B (g/l)	0.87 ± 0.18	0.87 ± 0.22	0.93 ± 0.24	0.579
Apo B/Apo A-I	0.59(0.53–0.7)	0.66(0.5–0.73)	0.57(0.49–0.7)	0.818

RA, reumathoid arthritis; HOMA-IR, homeostasis model assessment; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Apo, apolipoprotein.
Data are shown as mean ± SD or median (interquartile range) according to data distribution.
Differences were evaluated by ANCOVA using age as covariate.

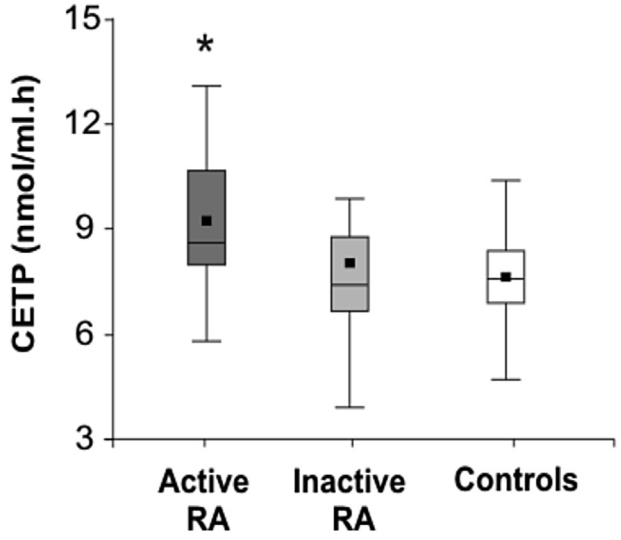

Fig. 1. Cholesteryl ester transfer protein (CETP) activity in RA patients, grouped according to disease activity score, and in healthy controls. $^{*}p = 0.026$ *vs.* inactive RA patients and controls. Tested by ANCOVA using age as covariate.

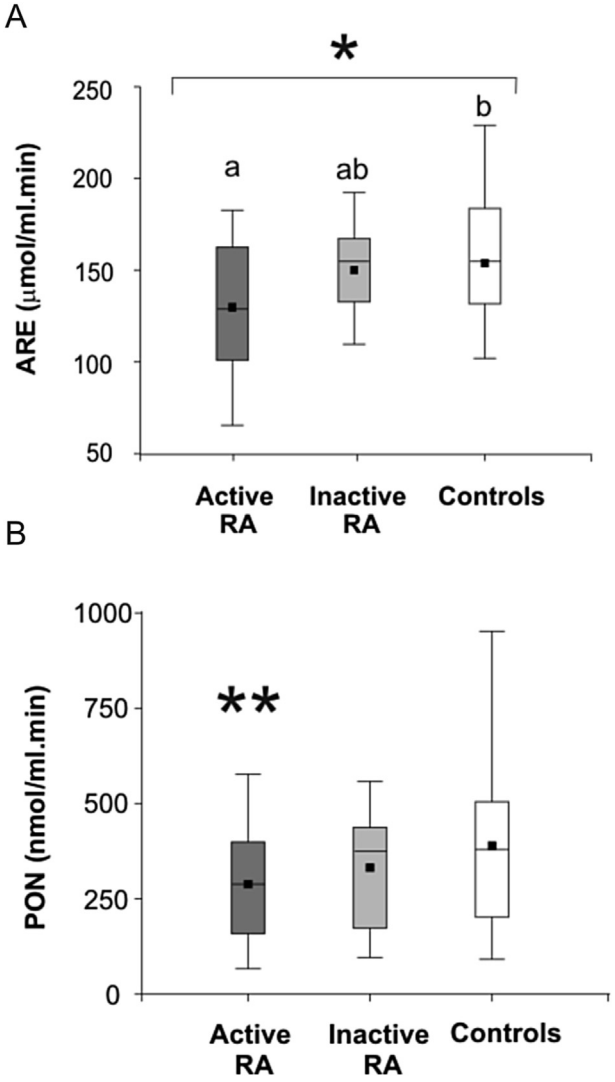

Fig. 2. Arylesterase (ARE) and paraoxonase (PON) activities of the enzyme paraoxonase 1 in RA patients, grouped according to disease activity score, and in healthy controls. (A) ARE activity of the enzyme paraoxonase 1, $^{*}p = 0.034$, tested by ANCOVA using age as covariate. Different letters depict significant differences between the groups according to Tukey's *post-hoc* test. (B) PON activity of the enzyme paraoxonase 1, $^{**}p = 0.041$, tested by ANCOVA with linear contrasts using age as covariate.

feature of RA involves elevated levels of the acute phase proinflammatory protein SAA [45,46]. However, in the present study, we were unable to detect differences in SAA levels between RA patients and controls, potentially reflecting the intense anti-inflammatory treatment been administered to the patients. Moreover, neither enzymatic activities of PON 1 nor concentrations of SAA were correlated with cf-PWV. Such observation further adds to the reports in which PON and ARE activities did not significantly improve cardiovascular risk prediction models [47].

CETP activity showed a negative correlation with plasma apo A-I levels in this series of RA patients. It has been shown that under conditions of elevated CETP activity composition alterations of the lipid core of HDL particles may lead to apo A-I dissociation from HDL [48]. Such mechanism is a well-documented phenomenon in individuals with atherogenic dyslipidemia and insulin resistance [15,16,49]. In addition, CETP activity was associated with waist circumference and triglyceride levels. Therefore, in active RA patients, besides inflammation, fat mass distribution can be contributing to elevated CETP activity. In contrast with our results, Ferraz-Amaro et al. [50] have previously reported low CETP activity in RA patients under treatment with glucocorticoids. In parallel, the authors have also documented elevated HDL-C levels, an observation that differs from our results and from data earlier reported by others [5]. In the group of active RA patients studied only 30% were under glucocorticoid treatment and CETP differences remained

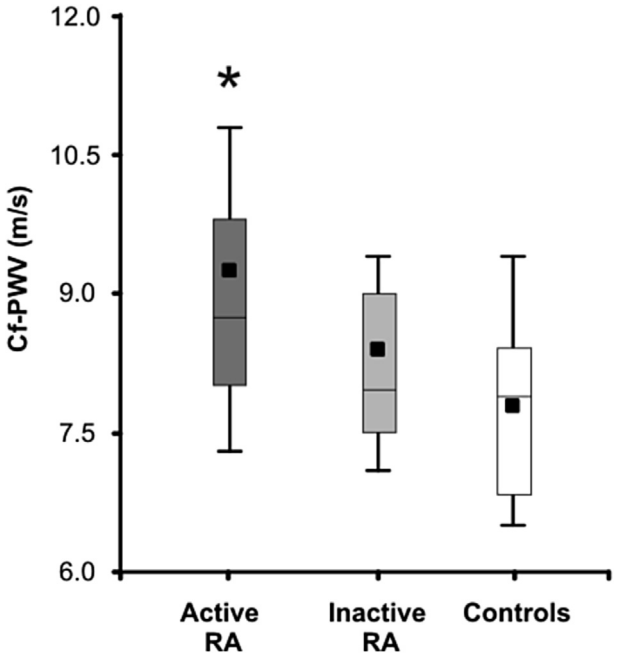

Fig. 3. Carotid to femoral pulse wave velocity (cf-PWV) in RA patients, grouped according to disease activity score, and in healthy controls. $^{*}p = 0.030$, tested by ANCOVA with linear contrasts using age as covariate.

Table 3
Multiple linear regression using carotid-femoral pulse wave velocity as dependent variable.

Variable	Bst	T	*p*	R^2
Age	0.468	2.730	0.013	0.42
hsCRP	0.143	0.816	0.425	
HOMA-IR	0.097	0.556	0.585	
Apo A-I	−0.405	−2.366	0.029	
ARE	−0.067	−0.390	0.701	

Bst, standardized beta coefficient; hsCRP, high-sensitivity C reactive protein; HOMA-IR, homeostasis model assessment; apo, apolipoprotein; ARE, arylesterase activity of PON 1.

significant even after adjustment by glucocorticoid use (data not shown). Therefore, the discrepancy between these studies can be attributed to the fact that glucocorticoid therapy, in fact, increases HDL-C levels in RA patients, probably by means of a reduction in CETP activity [51,52].

The present study reinforces the hypothesis that inflammation, acting through its effects at HDL, may further compromise the vascular health of patients with RA. Indeed, patients with inactive RA did not present any alteration of the HDL metrics assessed. Therefore, in normolipidemic RA patients who do not present insulin resistance, efficient control of inflammatory status should result in the maintenance of antiatherogenic functions of HDL.

The relatively low number of patients in the present study may have limited our capacity to detect subtle differences between the studied groups. In addition, carotid thickness was not evaluated in the present study and the relationship between HDL characteristics and the presence of atheromatous plaques was not assessed. By contrast, the differentiation between patients with high and low disease activity and the variety of HDL-related parameters evaluated are the major strengths of the study. Importantly, the group of patients evaluated showed no evident alterations in the lipoprotein profile as it is commonly observed in RA patients under anti-inflammatory treatment in the general practice. Therefore, the results of the present study suggest that the assessment of plasma apo A-I levels may be relevant in the clinical context and may contribute to improve RA care.

In conclusion, patients with a high DAS-28 score showed alterations in markers of HDL functionality as a consequence of chronic inflammation. Our data suggest that plasma levels of apo A-I can be used as a marker of arterial stiffness in RA patients. Longitudinal studies are required to confirm this important finding.

Conflict of interest

The authors declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

EB and TM evaluated the activity of the lipoprotein-associated enzymes, performed the statistical analyses and partially wrote the manuscript.

MM, WT, PS, MM and LB carried out the blood extractions, performed the general biochemical determinations and helped to carry out the recruitment.

VM and ES were responsible for the recruitment of both patients and controls.

CS and ES performed the pulse wave velocity measurements.

LGR, JC, AK and FB conceived the study, and participated in its design and coordination and helped to draft the manuscript.

All authors read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2016.06.009.

References

[1] H. Maradit-Kremers, C.S. Crowson, P.J. Nicola, et al., Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study, Arthritis Rheum. 52 (2005) 402–411.
[2] F. Wolfe, B. Freundlich, W.L. Straus, Increase in cardiovascular and cerebrovascular disease prevalence in rheumatoid arthritis, J. Rheumatol. 30 (2003) 36–40.
[3] N. Goodson, J. Marks, M. Lunt, D. Symmons, Cardiovascular admissions and mortality in an inception cohort of patients with rheumatoid arthritis with onset in the 1980s and 1990s, Ann. Rheum. Dis. 64 (2005) 1595–1601.
[4] I.D. del Rincon, K. Williams, M.P. Stern, G.L. Freeman, A. Escalante, High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors, Arthritis Rheum. 44 (2001) 2737–2745.
[5] A. Bag-Ozbek, J.T. Giles, Inflammation, adiposity, and atherogenic dyslipidemia in rheumatoid arthritis: is there a paradoxical relationship? Curr. Allergy Asthma Rep. 15 (2015) 497.
[6] E. Choy, K. Ganeshalingam, A.G. Semb, Z. Szekanecz, M. Nurmohamed, Cardiovascular risk in rheumatoid arthritis: recent advances in the understanding of the pivotal role of inflammation, risk predictors and the impact of treatment, Rheumatol. Oxf. 53 (2014) 2143–2154.
[7] A.V. Khera, M. Cuchel, M. de la Llera-Moya, et al., Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis, N. Engl. J. Med. 364 (2011) 127–135.

[8] A. Rohatgi, A. Khera, J.D. Berry, et al., HDL cholesterol efflux capacity and incident cardiovascular events, N. Engl. J. Med. 371 (2014) 2383–2393.
[9] P.J. Barter, K.A. Rye, The rationale for using apoA-I as a clinical marker of cardiovascular risk, J. Intern Med. 259 (2006) 447–454.
[10] M. Aviram, M. Rosenblat, C.L. Bisgaier, et al., Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase, J. Clin. Investig. (1998) 1581–1590.
[11] G. Baskol, H. Demir, M. Baskol, et al., Investigation of protein oxidation and lipid peroxidation in patients with rheumatoid arthritis, Cell Biochem. Funct. 24 (2006) 307–311.
[12] N. Tanimoto, Y. Kumon, T. Suehiro, et al., Serum paraoxonase activity decreases in rheumatoid arthritis, Life Sci. 72 (2003) 2877–2885.
[13] C. Popa, L.J. van Tits, P. Barrera, et al., Anti-inflammatory therapy with tumour necrosis factor alpha inhibitors improves high-density lipoprotein cholesterol antioxidative capacity in rheumatoid arthritis patients, Ann. Rheum. Dis. 68 (2009) 868–872.
[14] K.K. Ray, M. Ditmarsch, D. Kallend, et al., The effect of cholesteryl ester transfer protein inhibition on lipids, lipoproteins, and markers of HDL function after an acute coronary syndrome: the dal-ACUTE randomized trial, Eur. Heart J. 35 (2014) 1792–1800.
[15] J.A. de Souza, C. Vindis, B. Hansel, et al., Metabolic syndrome features small, apolipoprotein A-I-poor, triglyceride-rich HDL3 particles with defective anti-apoptotic activity, Atherosclerosis 197 (2008) 84–94.
[16] E. Nobecourt, S. Jacqueminet, B. Hansel, et al., Defective antioxidative activity of small dense HDL3 particles in type 2 diabetes: relationship to elevated oxidative stress and hyperglycaemia, Diabetologia 48 (2005) 529–538.
[17] P. Ambrosino, M. Tasso, R. Lupoli, et al., Non-invasive assessment of arterial stiffness in patients with rheumatoid arthritis: a systematic review and meta-analysis of literature studies, Ann. Med. 47 (2015) 457–467.
[18] A. Lo Gullo, G. Mandraffino, M.A. Sardo, et al., Circulating progenitor cells in rheumatoid arthritis: association with inflammation and oxidative stress, Scand. J. Rheumatol. 43 (2014) 184–193.
[19] K.M. Maki-Petaja, M. Elkhawad, J. Cheriyan, et al., Anti-tumor necrosis factor-alpha therapy reduces aortic inflammation and stiffness in patients with rheumatoid arthritis, Circulation 126 (2012) 2473–2480.
[20] S.A. Provan, K. Angel, A.G. Semb, et al., Early prediction of increased arterial stiffness in patients with chronic inflammation: a 15-year followup study of 108 patients with rheumatoid arthritis, J. Rheumatol. 38 (2011) 606–612.
[21] D. Aletaha, T. Neogi, A.J. Silman, et al., 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative, Ann. Rheum. Dis. 69 (2010) 1580–1588.
[22] J. Anderson, L. Caplan, J. Yazdany, et al., Rheumatoid arthritis disease activity measures: American College of Rheumatology recommendations for use in clinical practice, Arthritis Care Res. Hob. 64 (2012) 640–647.
[23] L. Lagrost, H. Gandjini, A. Athias, et al., Influence of plasma cholesteryl ester transfer activity on the LDL and HDL distribution profiles in normolipidemic subjects, Arterioscler. Thromb. 13 (1993) 815–825.
[24] C.E. Furlong, R.J. Richter, S.L. Seidel, L.G. Costa, A.G. Motulsky, Spectrophotometric assays for the enzymatic hydrolysis of the active metabolites of chlorpyrifos and parathion by plasma paraoxonase/arylesterase, Anal. Biochem. 180 (1989) 242–247.
[25] D.N. Nevin, A. Zambon, C.E. Furlong, et al., Paraoxonase genotypes, lipoprotein lipase activity, and HDL, Arterioscler. Thromb. Vasc. Biol. 16 (1996) 1243–1249.
[26] R. Asmar, J. Topouchian, B. Pannier, A. Benetos, M. Safar, Pulse wave velocity as endpoint in large-scale intervention trial. The complior study. Scientific, quality control, coordination and investigation Committees of the complior study, J. Hypertens. 19 (2001) 813–818.
[27] R. Asmar, A. Benetos, J. Topouchian, et al., Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies, Hypertension 26 (1995) 485–490.
[28] A. Isik, S.S. Koca, B. Ustundag, H. Celik, A. Yildirim, Paraoxonase and arylesterase levels in rheumatoid arthritis, Clin. Rheumatol. 26 (2007) 342–348.
[29] G. Baskol, H. Demir, M. Baskol, et al., Assessment of paraoxonase 1 activity and malondialdehyde levels in patients with rheumatoid arthritis, Clin. Biochem. 38 (2005) 951–955.
[30] P. Soltesz, H. Der, G. Kerekes, et al., A comparative study of arterial stiffness, flow-mediated vasodilation of the brachial artery, and the thickness of the carotid artery intima-media in patients with systemic autoimmune diseases, Clin. Rheumatol. 28 (2009) 655–662.
[31] G. Kocabay, H. Hasdemir, M. Yildiz, Evaluation of pulse wave velocity in systemic lupus erythematosus, rheumatoid arthritis and Behcet's disease, J. Cardiol. 59 (2012) 72–77.
[32] A. Arida, E. Zampeli, G. Konstantonis, et al., Rheumatoid arthritis is sufficient to cause atheromatosis but not arterial stiffness or hypertrophy in the absence of classical cardiovascular risk factors, Clin. Rheumatol. 34 (2015) 853–859.
[33] B. Garner, P.K. Witting, A.R. Waldeck, et al., Oxidation of high density lipoproteins. I. Formation of methionine sulfoxide in apolipoproteins AI and AII is an early event that accompanies lipid peroxidation and can be enhanced by alpha-tocopherol, J. Biol. Chem. 273 (1998) 6080–6087.
[34] B. Garner, A.R. Waldeck, P.K. Witting, K.A. Rye, R. Stocker, Oxidation of high density lipoproteins. II. Evidence for direct reduction of lipid hydroperoxides by methionine residues of apolipoproteins AI and AII, J. Biol. Chem. 273 (1998) 6088–6095.
[35] M. Kotosai, S. Shimada, M. Kanda, et al., Plasma HDL reduces nonesterified fatty acid hydroperoxides originating from oxidized LDL: a mechanism for its antioxidant ability, Lipids 48 (2013) 569–578.
[36] I. Baotic, Z.D. Ge, F. Sedlic, et al., Apolipoprotein A-1 mimetic D-4F enhances isoflurane-induced eNOS signaling and cardioprotection during acute hyperglycemia, Am. J. Physiol. Heart Circ. Physiol. 305 (2013) H219–H227.
[37] R.P. Dullaart, W. Annema, R.A. Tio, U.J. Tietge, The HDL anti-inflammatory function is impaired in myocardial infarction and may predict new cardiac events independent of HDL cholesterol, Clin. Chim. Acta 433 (2014) 34–38.
[38] K.P. Liao, M.P. Playford, M. Frits, et al., The association between reduction in inflammation and changes in lipoprotein levels and HDL cholesterol efflux capacity in rheumatoid arthritis, J. Am. Heart Assoc. 4 (2015).
[39] F. Montecucco, E. Favari, G.D. Norata, et al., Impact of systemic inflammation and autoimmune diseases on apoA-I and HDL plasma levels and functions, Handb. Exp. Pharmacol. 224 (2015) 455–482.
[40] G.T. Russo, A. Giandalia, E.L. Romeo, et al., Markers of systemic inflammation and Apo-AI containing HDL subpopulations in women with and without diabetes, Int. J. Endocrinol. 2014 (2014) 607924.
[41] M. Aviram, M. Rosenblat, Paraoxonases and cardiovascular diseases: pharmacological and nutritional influences, Curr. Opin. Lipidol. 16 (2005) 393–399.
[42] P. Kresanov, T. Vasankari, M. Ahotupa, et al., Paraoxonase-1 and oxidized lipoprotein lipids. The cardiovascular risk in Young Finns study, Atherosclerosis 241 (2015) 502–506.
[43] A. Zerrad-Saadi, P. Therond, S. Chantepie, et al., HDL3-mediated inactivation of LDL-associated phospholipid hydroperoxides is determined by the redox status of apolipoprotein A-I and HDL particle surface lipid rigidity: relevance to inflammation and atherogenesis, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 2169–2175.
[44] M. Mackness, B. Mackness, Human paraoxonase-1 (PON1): Gene structure and expression, promiscuous activities and multiple physiological roles, Gene 567 (2015) 12–21.
[45] C. Shen, X.G. Sun, N. Liu, et al., Increased serum amyloid A and its association with autoantibodies, acute phase reactants and disease activity in patients with rheumatoid arthritis, Mol. Med. Rep. 11 (2015) 1528–1534.
[46] L.G. Rosso, M. Lhomme, T. Merono, et al., Altered lipidome and antioxidative activity of small, dense HDL in normolipidemic rheumatoid arthritis: relevance of inflammation, Atherosclerosis 237 (2014) 652–660.
[47] S.K. Kunutsor, S.J. Bakker, R.W. James, R.P. Dullaart, Serum paraoxonase-1 activity and risk of incident cardiovascular disease: the PREVEND study and meta-analysis of prospective population studies, Atherosclerosis 245 (2016) 143–154.
[48] K.A. Rye, N.J. Hime, P.J. Barter, The influence of cholesteryl ester transfer protein on the composition, size, and structure of spherical, reconstituted high density lipoproteins, J. Biol. Chem. 270 (1995) 189–196.
[49] S. Rashid, T. Watanabe, T. Sakaue, G.F. Lewis, Mechanisms of HDL lowering in insulin resistant, hypertriglyceridemic states: the combined effect of HDL triglyceride enrichment and elevated hepatic lipase activity, Clin. Biochem. 36 (2003) 421–429.
[50] I. Ferraz-Amaro, M.A. Gonzalez-Gay, J.A. Garcia-Dopico, F. Diaz-Gonzalez, Cholesteryl ester transfer protein in patients with rheumatoid arthritis, J. Rheumatol. 40 (2013) 1040–1047.
[51] A.N. Georgiadis, E.C. Papavasiliou, E.S. Lourida, et al., Atherogenic lipid profile is a feature characteristic of patients with early rheumatoid arthritis: effect of early treatment–a prospective, controlled study, Arthritis Res. Ther. 8 (2006) R82.
[52] C. Garcia-Gomez, J.M. Nolla, J. Valverde, et al., High HDL-cholesterol in women with rheumatoid arthritis on low-dose glucocorticoid therapy, Eur. J. Clin. Investig. 38 (2008) 686–692.