TNF inhibitors exert a “hidden” beneficial effect in the cardiovascular lipoprotein profile of RA patients

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Abstract

BACKGROUND: Higher cardiovascular risk has been largely described in rheumatoid arthritis as well as different effects of biologic agents in theses patients. The aim of present study is to analyze the effects of tumor necrosis factor inhibitors (TNFi) in the lipoprotein profile of rheumatoid arthritis (RA) patients using a broad laboratory assessment including a large number of no routine tests

METHODS: RA patients treated with and without TNFi were cross-sectionally compared regarding a broad spectrum of lipoprotein tests including serum levels of cholesterol, triglycerides, HDL, LDL and VLDL cholesterol, lipoprotein A (LpA), apolipoprotein A1 (Apo A) and B**100** (Apo B), HDL, LDL and VLDL proteins, triglycerides and phospholipids, HDL Apo A, LDL and VLDL Apo B and paraoxonase 1 (PON 1), HDL, LDL and VLDL masses and number of particles of LDL and VLDL. Univariate and multivariate analyses of the different variables were performed

RESULTS: Patients on treatment with TNFi showed a trend to be younger and had a longer disease duration. Regarding to the lipoprotein analyses, borderline significant higher levels of serum Apo A were detected and an independent association with lower HDL mass, LDL triglyceride, VLDL cholesterol, VLDL Apo B, VLDL mass, number of VLDL cholesterol molecules and number of particles of VLDL was clearly observed.

CONCLUSIONS: TNFi treatment was associated with beneficial atherogenic effects at the lipoprotein level especially centered in VLDL related parameters consistent with a reduction of the remnant atherogenic risk.

Background

Rheumatoid arthritis (RA) is a multisystemic inflammatory condition but largely characterized by its articular involvement. However, during the last years, a large body of evidence has shown that this disease portends an increasing risk for cardiovascular complications(1, 2). Patients with RA have two- or three-fold higher risk than healthy people for suffering myocardial infarction and a 50% higher risk for stroke. They also have an increased incidence of cardiovascular disease (CVD) mortality with a standardized mortality ratio of 1.5 (95% CI 1.4 to 1.6) as it has been reported in several meta-analyses and systematic reviews(3–6). Furthermore, imaging studies have shown that these patients present an accelerated rate of atherogenesis in comparison with general population(7, 8).

The reasons for this increased risk of CVD in RA most probably include either traditional risk factors for CVD and disease related features(9). Patients with RA present a higher incidence of CVD risk factors such as lack of exercise, obesity, diabetes or dyslipidemia. In fact, changes in lipid profile, indicative of higher atherogenic risk, have been described even during the preclinical phase of this disease(10). These changes might be further worsened by some treatments as glucocorticoids. Furthermore, it has been described that the inflammatory and immunologic milieu of this type of diseases may exert pro-atherogenic effects at the endothelial and metabolic levels(11, 12).

Specifically, it has been reported that a systemic inflammatory status may have negative influence on lipid profile that can be visualized even before the clinical onset of the diseases, as it has been underscored above. Moreover, either in RA and in other immunologic mediated systemic diseases like systemic lupus erythematosus (SLE) it has been demonstrated that high-density lipoprotein (HDL) loss its protective character against atherogenesis turning out towards a pro-atherogenic phenotype(13). These lipoprotein alterations seem to be mostly mediated by some of the cytokines, which produce the inflammatory state characteristic of these diseases. To this regard TNFα, which has been seen to play a central role in the pathogenesis of RA, seems to have either important effects on lipid metabolism and atherogenesis (14). Furthermore, it has been reported that TNF is able to reduce the serum activity of paraoxonase 1 (PON 1) being this enzyme one of the main factors that mediates the anti-atherogenic activity of HDL(15, 16).

Treatment of RA has been improved a lot during the last decades, not only due to the development of highly effective targeted agents but also because of an earlier and more aggressive therapeutic approach based in a treat to target philosophy. This stricter control of the inflammation should also lead to a better outcome in the disease-related CVD complications. In fact, the use of methotrexate has been associated with a decrease in CVD mortality(17).

Biologics agents and specifically anti-TNF therapy have been a step forward in the management of this disease. Undoubtedly, they have allowed better control of the clinical disease activity using a more specific and targeted mechanism of action. Although, paradoxical effects of the TNF inhibitors (TNFi) on the serum levels of cholesterol and main lipoproteins such as LDL and HDL, beneficial effects in the outcome of the CVD events in RA have been reported at the clinical level(18). Whether this positive effect is only due to the better inflammatory control or these agents may exert a specific beneficial action at the metabolic level has not been totally cleared up

The objective of our study is to analyze the effects of TNFi on the lipoprotein metabolism studying in-depth changes in the lipoprotein profile of these patients.

Methods

STUDY DESIGN AND SETTING

Cross-sectional comparative study of patients on treatment with TNFi or only with conventional synthetic DMARDs (csDMARDs). The study has been undertaken in two University hospitals (Hospital Universitario Marqués de Valdecilla and Hospital Universitario Sierrallana) of the autonomic community of Cantabria, northern Spain, covering a total health area about half a million inhabitants.

Our study was approved by our regional IRB committee named: *Comité de Etica e Investigación de Cantabria*. All procedures of the present study were performed in agreement with the principles of World Medical Association's Declaration of Helsinki.

POPULATION TO STUDY

Patients older than 18 years and fulfilling the 1987 revised ACR classification criteria for rheumatoid arthritis (RA) were eligible for the study. Patients had to be on treatment with TNFi at least during the last six months (TNFi group). A 1:1 sex and age matched group of patients treated only with csDMARDs was also selected (csDMARD group) (19).

GENERAL CLINICAL EVALUATION

In all the patients a medical chart review was done and clinical, laboratory and comorbidity data were recorded. Physical and complete joint examination was performed as well. Then, all the participants were assessed at the outpatient clinic once. Blood tests were obtained including blood count cells, biologic markers of clinical activity as erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) and immunology tests as ANA, rheumatoid factor (RF) or anti-citrullinated protein antibodies (ACPA). Additionally, blood samples were sent to the central laboratory for this study (cardiovascular risk laboratory at the Hospital Universitario Marqués de Valdecilla) for performing all the specific lipid determinations for this study (see below).

CLINICAL ACTIVITY AND DISCAPACITY ASSESSMENT

To assess clinical activity the investigators calculated in all the cases the DAS 28 (ESR) index (20). This internationally validated and standardized index collapses, using a mathematical formula, diverse clinical, laboratory patient reported parameters as the number of tender and swollen joints, the patient global assessment using a visual analogic scale from 0 (perfect state) to 100 (the worst possible state) and ESR, obtaining a numeric value which represent the level of clinical activity of the disease This index score allow to classify the patients in remission (< 2.6), low disease activity (≥ 2.6, < 3.2), moderate disease activity (≥ 3.2, < 5.1) and high disease activity (≥ 5.1).

Disability was assessed using the Health Assessment Questionnaire (HAQ)(21). This questionnaire evaluates this issue asking about the difficulty for performing specific daily activities grouped in eight blocks. Each question scores from 0 to 3 depending on the level of difficulty that the patient refers for doing such activity (0, none difficulty, 1 some, 2 much or 3 impossible of doing it) being the final score of each block that of the worst answer. The final index score goes from 0 to 3, resulting from the sum of eight blocks divided by eight.

SPECIFIC LIPID AND ADDITIONAL REALTED PARAMETERS

Plasma and serum samples were obtained by centrifugation at 1500 g, aliquoted, and frozen at -80 °C, in lower than 30 minutes after blood collection. In total serum were determined: total cholesterol, triglycerides and total proteins by automatized enzymatic methods (ADVIA 2400 Clinical Chemistry System, Siemens Healthcare Diagnostics, Newark, United States), phospholipids (BEN Biochemical Enterprise, Italy) and HDL cholesterol (HDL-C) by enzymatic method after precipitation of non-HDL lipoproteins with phosphotungstic-magnesium acid of Biosystems (Barcelona, Spain) by slightly

modified CHOD-PAP method, in a Dimension RXL- Siemens Healthcare Diagnostics), apolipoprotein A1 (Apo A), apolipoprotein B100 (Apo B), high sensitivity CRP (hsCRP) and Serum Amyloid A (SAA) by inmunonephrelometry (BNII-System, Siemens Healthcare Diagnostics, Germany), paraoxonase 1 (PON1) (Molecular Probes, UK), and activity of secretory phospholipases A2 (sPLA2) (Cayman Chemical, USA).

Separation of lipoproteins: a combined ultra-centifugation method was used: the first centrifugation (40 minutes to 13500 rpm, 5424 Eppendorf centrifuge) for separating chylomicrons and the second ultracentrifugation of the previous infranadant in density gradient (1,006–1,300 g/ml, dynamic gradient with potassium bromide (KBr)) for separating VLDL, LDL and HDL, in Optima L-90 Beckman Coulter (23 hours to 57.000 rpm). In all the obtained subfractions (after dialysis with saccharose-PBS-EDTA-Azida) cholesterol, triglycerides, phospholipids, proteins (colorimetric assay, coomassie brilliant blue -CBB R250, Sigma, USA) and potassium concentration were measured. In addition, in the HDL subfraction the mean density was measured (through the concentration of KBr used for the formation of the gradient previously mentioned), as well as the concentration of specific proteins like ApoA1, SAA and PON 1.

In all the subfractions the final volume was corrected to be referred to its specific concentration in whole serum (measured volume through the weight/ density rate and referred to the initial used serum volume).

The number of VLDL and LDL particles was estimated from Apo B determination by their equimolar presence (1 particle of Apo B in each one of these two fractions). The number of cholesterol and triglyceride molecules per particles was estimated from the determination of cholesterol and triglycerides for each fraction. Total mass of each lipoprotein was calculated by adding the estimated masses for each fraction of cholesterol, triglycerides, phospholipids and proteins.

STATISTICAL METHODS

Our initial sample size estimation was to include, at least 70 patients per group. There no exist much information in the literature regarding data explored in our study but accordingly to some studies which have approached the study of some of these parameters (13), to detect the expected differences in them with a level of two tailed significance of 5% and a power of 80%, and assuming similarity between the standard deviations of the groups to be compared, forty patients, at least, would have been necessary for each group. Anticipating a 20% of loses, we finally though that the number of patients aforementioned by group would be enough to carry out the study.

Standard descriptive parameters were used for showing salient characteristics of the patients. For univariate analysis, Chi-square for categorical variables and Student' T test for continuous variables were applied. In the case that the distribution of the continuous variable was not normal non-parametric tests Mann-Whitney´U test was utilized.

Variables of interest found to differ significantly between both subsets of patients were further analyzed. In order, to control potential confounding factors, multivariate linear regression models were built. In all the cases, all the variables found to be significant in the univariate analysis as well as those to be thought clinically relevant were entered in the different multivariate models using a manual backward

variable selection strategy according to the level of significance of each variable to select the most parsimonious and biological consistent models.

SPSS software V.23 (IBM SPSS Statistics Armonk, NY, USA) was used for all statistical analyses.

Results

GENERAL FINDINGS

We finally enrolled seventy patients in each group. In Table 1 demographics and salient clinical and therapeutic characteristics of both subsets are shown. The total population represents the typical RA cohort with higher female percentage and with percentages of positivity for rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) between sixty and seventy percent (61.2 and 70.5% respectively), without significant inter-subset differences in all these figures. Mean age at diagnosis and disease duration were 51.1 ± 13.1 years old and 99.8 ± 76.8 months for the entire cohort. Patients in the TNFi subgroup had a trend towards younger age (49.0 ± 12.5 vs 53.2 ± 13.3 years old; p = 0.058) and a significant longer disease duration (117.5 ± 77.3 vs 81.6 ± 72.1; p = 0.005). These data most probably reflects two facts, the bias of using biologics in younger patients and the use of these agents in patients with longer follow up after having tried csDMARDs. No relevant differences were found between the two subgroups of patients regarding to the comorbidities recorded in the study.

With respect to the clinical status of the patients, biologic and composite indices showed that patients in the csDMARD subgroup presented higher disease activity with greater values of ESR (40.7 ± 26.0 vs 28.3 ± 4; p = 0.025), CRP (10.7 ± 18.5 vs 2.3 ± 5.9; p = 0.001) and DAS 28 (4.0 ± 1.4 vs 3.6 ± 1.2 p = 0.036). Conversely, the level of functional impairment as assessed by HAQ was similar in both subsets of patients.

Finally, with respect to treatment the large majority of patients were still on csDMARDs, mostly using methotrexate. A larger percentage of patients on TNFi therapy were off any type of csDMARD (29.4 vs 3.1%; p = < 0.001).

LIPOPROTEIN PROFILE COMPARISONS: UNIVARIATE ANALYSES

A detailed description of the univariate comparisons of the all parameters analyzed is depicted in Table 2. No differences between both groups of patients were observed in the standard lipid profile (concentrations of total cholesterol, HDL and LDL-Cholesterol and triglycerides). We did not find either, differences in the serum total concentrations of Lipoprotein (a) [Lp (a)] or Apo B but a borderline significant difference with a higher value of Apo A in the TNFi subset was observed (169.8 ± 29.3 vs 160,2 ± 28.2; p = 0.052).

Regarding to the specific assessments of each lipoprotein we find the following results. In HDL, no differences were observed in the composition of the different lipid constituents (cholesterol, triglycerides and phospholipids). For the contrary a decrease in the protein concentration was present in the TNFi subgroup (1027.5 ± 488.3 vs 1393.3 ± 451.1; p = < 0.001). Likewise, a lower HDL mass was recorded among the patients with TNFi (2838.2 ± 887.2 vs 3341.2 ± 887.4; p = 0.001). On the other hand, no significant differences were found in any parameter related to LDL with the exception of triglyceride subfraction concentration (22.7 to 28.6; p = 0.003). Finally, the most noticeable findings were seen in relation with the VLDL particle. Patients treated with TNFi showed lower levels of cholesterol, phospholipids and proteins including Apo B, a decrease in VLDL total mass as well as in the number of particles of VLDL.

MULTIVARIATE ANALYSES

We did build different multivariate models for all those variables that had shown statistically significant inter group differences and with relevant biologic meaning for the objective of the study, entering in each case all the possible confounding variables to explore the independent association with the utilization of TNFi. As it can be observed in Table 3, such independent association was corroborated in all the cases, regardless that different additional variables had also an independent association with the dependent variable in some of the cases. Specifically, to note that in the majority of VLDL parameters including decrease in cholesterol, Apo B, total mass and number of particles levels, in addition with the treatment with TNFi the use of statins showed an independent effect as well.

Discussion

Our results show that TNFi produce a net beneficial effect in the lipoprotein profile of RA patients which may lead to a lower atherosclerotic risk. However, this biochemical effect is generally overlooked given that their effects seem to be centered in several aspects related with VLDL particles which are not routinely determined.

The VLDL particles are produced by the liver and are triglyceride rich. They contain various apolipoprotein and Apo B-100 is the core structural protein, each VLDL particle contains one Apo B-100 molecule. These nascent VLDL are in charge of transporting these lipids from the liver to the peripheral tissues where they suffer the lipolysis of the triglycerides(22, 23). The nascent VLDL particles do not portend a high atherogenic risk since they have a low content in cholesterol and a bigger molecular size than other lipoproteins, which makes difficult that these molecules can go through the endothelial cell barrier into the arterial walls. At the peripheral level, the triglyceride depletion of VLDL produces remnant VLDL molecules of smaller size and enriched in cholesterol concentration. Some of these particles are transformed into the LDL by the action of enzymes as hepatic lipase (HL) which totally depletes them from triglycerides and cholesteryl ester transfer protein (CEPT) that allows them to capture esterified cholesterol molecules(24, 25). On the other hand, part of the remnant VLDL remain in the circulation becoming highly atherogenic particles. A body of evidence exists pointing out an important role of Triglyceride-rich lipoproteins, and especially VLDL in cardiovascular risk (26, 27). Moreover, some recent

studies have shown that this subfraction of lipoprotein is especially relevant for the cardiovascular risk in those individuals with LDL level below of 130 mg/dl, which is part of remnant cardiovascular risk(28).

According to the data of our study, RA patients on TNFi therapy present lower levels of atherogenic VLDL related parameters including the concentration of total VLDL-cholesterol, VLDL-proteins and specifically VLDL-Apo B, total VLDL mass, number of cholesterol molecules per VLDL particle and number of VLDL particles. Besides, regarding the last-mentioned parameter, it has been underscored that the number of particles is a more accurate measurement than the cholesterol lipoprotein concentration to assess the cardiovascular risk. A higher number of particles is usually associated with a smaller molecular size and the smaller the lipoproteins are the more atherogenic they become(29).

Regarding the rest of main lipoproteins our study showed less pronounced effects. HDL is a lipoprotein which consists of a core of hydrophobic lipids, including cholesteryl esters and triglycerides, and a surface monolayer containing phospholipids, free cholesterol, and apolipoproteins(30). Apo A is, largely, the most abundant protein, representing 70% of the total. In addition, HDL contain the enzyme PON 1 that is responsible for the antioxidant properties of the HDL. Thus, this lipoprotein exerts anti-inflammatory and athero-protective effects in healthy individuals due to its capacity of removing the excess cholesterol from macrophages of the arterial wall as well as preventing LDL particles of being oxidized(31). However, in several chronic inflammatory conditions such as RA, HDL may turn out into a dysfunctional state losing these protective functions and shifting to a more proinflammatory phenotype(13). In these situations, it has been observed that Apo A is displaced by SAA, that is elevated in chronic inflammatory states, and at the same time is largely oxidized, whereas there is also a decrease of PON activity. All these changes lead to a minor cholesterol efflux activity and a lower anti-oxidative capacity(32, 33). We did not assess functional HDL activity in our study and therefore we cannot know whether TNFi help to reverse the potential proinflammatory phenotype of HDL. Nevertheless, we did not observe major changes in the HDL composition. Our results only showed a significant decrease in HDL mass mainly due to a decrease in the protein content. We may speculate that this protein depletion might be due to a decrease in the HDL-SAA concentration given that we did not see significant differences in the HDL-Apo A levels (the other preponderant protein). Moreover, we observed that TNFi associate a borderline significant increase in the Apo A serum levels along with a no significant trend (by parametric analysis) to lower serum levels of SAA; in fact when a non-parametric analysis as Mann Whitney U test was performed, given the distribution skewness of this variable, the difference of serum SAA became significant ($p = 0.001$; data not showed). On the other hand, we did not observe differences in the serum concentration of PON (data not shown), although no anti-oxidative capacity specific tests were performed.

As it has been stated, LDL particles are produced from the VLDL. They are rich in cholesterol and Apo B is their main protein(34) and carry the highest risk for atherosclerosis. These particles form a relatively heterogeneous population of lipoproteins with different sizes and density being those smaller and denser more atherogenic. According our results, the only relevant change observed in the TNFi subset of patients in the LDL composition was a significant increase of triglycerides. Although, the triglyceride enrichment of theses particle might be associated to less penetration and deposit of particles under endothelium, and

consequently a decrease in the cardiovascular risk, this fact has not yet clearly established (35). We did also observe a non-significant increase in the number of particles in patients treated with TNFi, which is difficult to interpret at this moment.

Finally, we measured serum levels of Lp (a) other cholesterol rich and atherogenic lipoprotein, but no significant differences were found. Lp (a) serum level are genetically determined and minor changes are expected through an individual's lifetime, except for acute inflammatory states. Treatment with statins do not modify circulating levels of Lp(a), and only recently some data have been published notifying a decrease in Lp(a) concentration in patients treated with PCSK9 inhibitors. Our results suggest that treatment with TNFi, despite their effect on the inflammatory state, do not seem to alter Lpa levels (36).

We recognize that our study has several limitations derived from its transversal design. However, we have tried to overcome in part such problems using multivariable models for adjusting by potential confounders as demographic factors, disease activity, or use of statins for example. The relatively limited sample size can also pose some concerns, especially when multiple comparisons are performed. Again, we think that the use of multivariate models may circumvent somehow this issue. Finally, as it has been mentioned functional analysis of HDL oxidative capacity are lacking but we focus our study in an in-depth analysis of the composition and structure of the main lipoprotein using determinations that to our knowledge has not been previously reported in these types of patients.

In conclusion, we are reporting that in RA patients treatment with TNFi produce beneficial effects at the lipoprotein level and from an atherogenic point of view with triglycerides enrichment of the LDL particles and, mainly, smaller remnant atherogenic risk with lower VLDL-cholesterol, VLDL-Apo B, total VLDL mass, number of cholesterol molecules per VLDL particle and number of VLDL particles. All this positive effect remains hidden using conventional lipid determinations and may provide some mechanistic explanations for the cardioprotective effects that these agents have shown in clinical registries.

Declarations

Ethics approval and consent to participate

As stated in the methods section our study was approved by our regional IRB committee named: *Comité de Etica e Investigación de Cantabria* an all procedures of were performed in agreement with the principles of World Medical Association’s Declaration of Helsinki.

Consent for publication

No individual person’s data in any form is included in the present work. All participants in the present study signed an informed consent

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

No author has any specific disclosure to declare.

Founding

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Authors' contributions

JCA conceived of the study and it was designed in collaboration with JGG (this author passed away during the preparation the present manuscript). BAL and ARGR participated mainly in the laboratory tasks and EAA and VMT in the patient recruitment and assessment. All authors contributed to refinement of the study protocol and approved the final manuscript.

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Tables

Table 1: General Characteristics

	Total	csDMARD	Anti-TNF	p
Age at diagnosis, years (mean±SD)	51.1±13.1	53.2±13.3	49.0±12.5	0.058
Gender (%)	77.9	77.1	78.6	1.00
Disease duration, months (mean±SD)	99.8±76.8	81.6±72.1	117.5±77.3	0.005
Smoking (%)	21.4	22.9	20.0	0.837
Comorbidities (%)				
▪ High blood pressure	35.7	35.7	35.7	0.603
▪ Dislipemic disorder	36.4	37.1	35.7	0.587
▪ Cardiovascular disease	13.6	15.7	11.4	0.448
▪ Diabetes	10.1	13.0	7.1	0.274
▪ Osteoporosis	8.6	1.4	7.2	0.031
Rheumatoid factor (%)	61.2	63.8	58.6	0.325
ACPA (%)	70.5	66.7	74.3	0.212
ANA (%)	33.3	43.1	23.4	0.025
Hemoglobin, mg/dL (mean±SD)	13.3±1.4	13.2±1.5	13.4±1.4	0.373
ESR, mmHg (mean±SD)	34.1±24.1	40.7±26.0	28.3±20.2	0.002
CRP, mg/L (mean±SD)	6.8±14.3	10.7±18.5	2.3±5.9	0.001
DAS28 (mean±SD)	3.78±1.3	4.02±1.4	3.6±1.2	0.036
HAQ (mean±SD)	0.58±0.53	0.57±0.51	0.59±0.56	0.875
csDMARD (current)				<0.001
▪ MTX	57.1	56.9	57.4	
▪ LEF	22.6	32.3	13.2	
▪ Otros	1.5	3.1	0.0	
▪ COMBO	2.3	4.6	0.0	
Anti-TNF (type, %)	NA	NA		NA
▪ Infliximab			35.7	
▪ Etanercept			41.4	
▪ Adalimumab			21.4	
▪ Golimumab			0.0	
▪ Certolizumab			1.4	
Glucocorticoids (GCs) (%)	42.6	52.2	33.3	0.037
Statins (%)	65.7	65.7	65.7	1.00

ACPA: Anti-citrullinated peptide antibody; ANA: Anti-nuclear antibody; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate

TABLE 2: Univariate analyses

TABLE 3: Multivariate analyses (best models)

	csDMARD	Anti-TNF	p
Serum SAA (mean±SD)	27.5±83.5	16.4±36.5	0.330
Serum Liproteína A (mean±SD)	28.8±28.0	22.9±29.9	0.233
Serum Apo A (mean±SD)	160.2±28.2	169.8±29.3	0.052
Serum Apo B (mean±SD)	87.8±20.6	88.6±14.9	0.796
Serum cholesterol (mean±SD)	204.8±36.5	204.2±34.5	0.921
Serum triglycerides (mean±SD)	97.9±38.8	92.7±48.2	0.489
HDL assessments, (mean±SD)			
	65.1±20.8	64.2±21.0	0.800
▪ HDL-Cholesterol, mg/dL	19.0±5.6	19.3±9.5	0.784
▪ HDL- Triglycerides, mg/dL	107.3±35.0	97.9±34.9	0.114
▪ HDL- Phospholipids, mg/dL	1393.3±451.1	1027.5±488.3	<0.001
▪ HDL-Proteins, mg/L	150.1±55.5	134.6±35.2	0.061
▪ HDL-Apo A	3341.2±887.4	2838.2±887.2	0.001
▪ Mass, mg/L			
LDL assessments, (mean±SD)			
	115.9±0.928	120.7±30.7	0.355
▪ LDL-Cholesterol, mg/dL	22.7±6.4	28.6±14.8	0.003
▪ LDL-Triglycerides, mg/dL	89.3±28.8	92.4±30.9	0.548
▪ LDL-Phospholipids, mg/dL	383.5±105.1	404.5±114.6	0.261
▪ LDL-Proteins, mg/L	2660.7±601.9	2816±698.0	0.160
▪ Mass, mg/L	2259.1±830.6	2080.9±380.4	0.107
▪ Nº of molecules of LDL-Cholesterol	201.5±5	219.4±106.2	0.280
▪ Nº of molecules of LDL-Triglycerides	837187.0±235908.6	902202.6±18357.4	0.056
▪ Nº of particles			
VLDL assessments, (mean±SD)			
	23.8±11.6	19.3±11.4	0.023
▪ VLDL-Cholesterol, mg/dL	56.3±33.4	45.1±42.8	0.088
▪ VLDL-Triglycerides, mg/dL	26.0±13.6	19.8±17.7	0.021
▪ VLDL-Phospholipids, mg/dL	292.7±529.0	72.3±49.0	0.001
▪ VLDL-Proteins, mg/L	1391.9±774.8	906.7±707.6	<0.001
▪ Mass, mg/L	3115.4±892.3	3567.1±1296.3	0.018
▪ Nº of molecules of VLDL-Cholesterol	3224.2±1542.7	3501.5±2178.4	0.367
▪ Nº of molecules of VLDL-Triglycerides	139489.4±140826.1	90194.3±56733.9	0.008
▪ Nº of particles			
Atherogenic index	3.4±1.1	3,4±1.0	0.841
Serum Paraoxonase, U/ml (mean±SD)	52.4±22.5	44.7±22.0	0.063

	R^2	Selected variables	p
HDL-Proteins	0.183		0.024
		▪ TNFi	0.082
		▪ Age	0.104
		▪ Disease duration	0.962
		▪ GCs	
HDL-Mass	0.142		<0.001
		▪ TNFi	0.072
		▪ Gender	0.189
		▪ Smoking	0.189
		▪ Disease duration	0.188
		▪ Statins	
LDL-Triglycerides	0.133		<0.001
		▪ TNFi	0.003
		▪ Age	0.227
		▪ GCs	
VLDL-Cholesterol	0.095		0.021
		▪ TNFi	0.026
		▪ Statins	0.289
		▪ Gender	0.574
		▪ GCs	
VLDL-Apo B	0.119		0.003
		▪ TNFi	0.043
		▪ Statins	0.191
		▪ DAS 28	0.628
		▪ GCs	
VLDL-Proteins	0.131		0.001
		▪ TNFi	0.080
		▪ Gender	0.117
		▪ DAS 28	0.321
		▪ Statins	0.572
		▪ Disease duration	
VLDL-Mass	0.219		<0.001
		▪ TNFi	0.022
		▪ Statins	0.030
		▪ DAS 28	0.045
		▪ Gender	0.079
		▪ Disease duration	
VLDL-nº Particles	0.094		0.004
		▪ FAME vs TNFi	0.019
		▪ Statins	0.452
		▪ GCs	