Increased fasting apolipoprotein B48 in rheumatoid arthritis: A report of the FRANCIS study

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Abstract

Background and aims: Serum concentrations of apolipoprotein (apo) B48, the structural protein of chylomicrons, are increased in conditions associated with systemic inflammation. As such, apo B48 may help to identify patients at increased cardiovascular risk. The objective of this study was to evaluate apo B48 levels in relation to other cardiovascular risk factors in patients with rheumatoid arthritis (RA).

Methods: We performed a prospective cohort study with RA patients without clinical cardiovascular disease or diabetes mellitus. Blood samples were collected after an overnight fast and a complete lipid profile, including total serum apo B and apo B48 (ELISA), was measured.

Results: 328 Patients were included. The mean age was 53±11 years and 224 patients (68%) were female. Remnant cholesterol (remnant-C) concentration was 0.52±0.26 mmol/L and fasting plasma triglycerides 1.25±0.88 mmol/L Median plasma apo B48 was 8.6 [IQR 5.2-12.5]. Serum apo B48 correlated positively with triglycerides ($r=0.651$; $p<0.001$, remnant-C ($r=0.479$; $p<0.001$ and LDL-C ($r=0.123$; $p=0.03$). Patients in the highest apo B48 tertile were, compared to the lowest tertile, more often rheumatoid factor positive 75% [n=72] vs. 58% [n=62]; $p=0.04$) and anti-CCP positive (75% [n=62] vs. 59% [n=59]; $p=0.005$).

Conclusion: RA patients have a high level of apo B48, despite low plasma triglycerides and remnant-C, which indicates that chylomicron remnant clearance may be delayed in RA, especially in rheumatoid factor or anti-CCP positive subjects.

Introduction

The evidence on the increased cardiovascular disease (CVD) risk associated to rheumatoid arthritis (RA) has accumulated during the last two decades. The prevalence of CVD in patients with RA is as high as in patients with type 2 diabetes mellitus (T2DM) (1, 2). Traditional cardiovascular risk factors, such as hyperlipidemia and hypertension, as well as RA specific risk factors, such as RA disease activity, erythrocyte sedimentation rate and C-reactive protein (CRP) levels contribute to the total CVD risk of RA patients (3-6).

Postprandial hyperlipidemia with accumulation of remnants has gained interest in the literature because of recent reports showing that non-fasting triglycerides are independent predictors of the risk of atherosclerosis (7, 8). Postprandial hyperlipidemia is often present in patients with a high CVD risk, such as patients with established coronary artery disease (CAD), T2DM (9, 10), the metabolic syndrome (11, 12), obesity (13) and familial combined hyperlipidemia (FCH) (14). Moreover, postprandial hyperlipidemia is closely associated to the generation of atherosclerosis (15-17). Postprandial chylomicrons and their remnants are able to induce leukocyte activation and can be internalized by macrophages without the need for prior modification, causing foam cell formation (18) and therefore, initiate atherosclerosis (19).

Postprandial hyperlipidemia is usually the consequence of hepatic very low-density lipoprotein (VLDL) overproduction and delayed clearance of chylomicrons and VLDL with their respective remnants since they share the same metabolic pathway (11, 14, 20, 21). In T2DM, intestinal overproduction of chylomicrons has been demonstrated, which also contributes to the postprandial hyperlipidemia typical of this disorder (22). Overall, these metabolic processes lead to elevated concentrations of chylomicrons and VLDL, and their respective remnants, collectively known as triglyceride-rich lipoproteins (TRLs). All these atherogenic lipoproteins contain apolipoprotein (apo) B as structural protein. In humans, two forms of apo B exist: apo B100, which is found on hepatically derived lipoproteins, and apo B48, which is present on the intestinally derived chylomicrons and their remnants (23). Therefore, plasma apo B48 levels represent the exact number of circulating chylomicrons and chylomicron remnants.

Apo B48 levels are closely associated to postprandial lipoprotein metabolism, even in the fasting state, and elevated levels of fasting apo B48 reflect postprandial hyperlipidemia (24). Moreover, fasting apo B48 is positively associated with several cardiovascular risk factors, including plasma triglycerides, remnant-cholesterol (remnant-C), body mass index (BMI) and carotid intima-media thickness (cIMT), a surrogate marker for subclinical atherosclerosis, and it correlates negatively with high-density lipoprotein cholesterol (HDL-C) (25, 26).

To date, no data on apo B48 or postprandial lipemia in RA are available. The chronic inflammatory state in RA may be associated to impaired chylomicron (remnant) me-

tabolism, which in turn may contribute to the increased risk of CVD in these patients. Therefore, we aimed to evaluate apo B48 levels in RA in relation to other cardiovascular risk factors.

Materials and methods

Participants

RA patients in this study were participants in the FRANCIS (Franciscus Rheumatoid Arthritis and Cardiovascular Intervention Study) trial. The FRANCIS study is an open label randomized clinical trial in which RA patients younger than 70 years old and without current CVD or T2DM are routinely screened for traditional CVD risk factors and, based on randomization, strictly treated for traditional CVD risk factors, at the Diabetes and Vascular Center of the Franciscus Gasthuis, Rotterdam, the Netherlands (27, 28). CVD was defined as a prior myocardial infarction, cerebrovascular event, amputation due to peripheral artery disease, intermittent claudication, percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass graft (CABG). T2DM was defined as fasting glucose > 7.0 mmol/l. The data for this study are the baseline measurements in the FRANCIS study (registered at trialregister.nl, number NTR3873).

Approval was given by the Institutional review board of the Franciscus Gasthuis and the regional independent medical ethical committee at the Maasstad Hospital in Rotterdam. All participants gave written informed consent.

Data collection

The medical history, anthropometric measures and the use of medication were recorded.

RA disease activity was assessed by using the Disease Activity Score with 28 joints counted (DAS28). This score included swollen joint count (28), tender joint count (28), VAS (0-100 scale) indicating pain/discomfort due to RA, and the erythrocyte sedimentation rate (ESR).

The estimated cardiovascular risk was assessed using the SCORE tables, and included suggested adaptations according to EULAR 2015 recommendations. The EULAR recommendation implies a multiplication of the CVD risk by 1.5 for all RA patients (29).

Blood samples were taken after an overnight fast of at least 12 hours and included a complete lipid profile with both total plasma apo B and apo B48.

Analytical Methods

Basic parameters for renal and liver function as well as glucose, total cholesterol, HDL-C and triglycerides were determined using a Synchron LX-20 or DxC analyzers (Beckman Coulter, Brea CA, USA) according to standard procedures in our laboratory for clinical

chemistry. LDL-C values were calculated using the Friedewald formula. Remnant-C was calculated by subtracting LDL-C and HDL-C from the total cholesterol. Apo AI and apo B were determined by rate nephelometry using an IMMAGE analyzer (Beckman Coulter, Brea CA, USA). The ESR was measured using an Alifax Test 1 analyzer (Alifax, Padova, Italy).

Apo B48 serum levels were quantified as previously reported (30), using a commercially available ELISA (Shibayagi Co., Ltd. Japan), consisting of a Sandwich type ELISA with absorbance dichromatic reading at 450 nm/ 620 nm (reference for plate correction) wavelength (31). All samples were assayed in three runs. Since no commercial quality controls are available for apo B48, a local internal quality control was pooled according to WHO recommendations, stored at - 80 ^{o}C, and formerly assayed by duplicate on each plate in parallel to samples. Calculated Variation Coefficients (VC) were 5.7% (intra-assay), 11.0% (inter-assay) and 12.4% (total).

Statistics

Data are given as mean ± standard deviation (SD) unless stated otherwise. Correlation analysis was performed using a Pearson's correlation. For comparison of patients with relatively high and low apo B48 levels, tertiles were created. Analyses were performed between the lowest and the highest tertile. Analyses were performed using the Students t-test or chi-square test where applicable. For skewed variables (DAS28, C-reactive protein, triglycerides) the Mann-Whitney U test was used. All statistical analyses were carried out using PASW statistics version 18.0 (IBM SPSS Statistics, New York, United States). P-values below 0.05 (two sided) were considered statistically significant.

Results

General characteristics

In total, 328 subjects with RA were included. The general characteristics are shown in Table 1. Most patients were female (n=224; 68%) and their age was 53 ± 11 years. The median RA disease duration was 7 [IQR 2-14] years. The median DAS28 was low, indicating low RA disease activity or remission. Most RA patients (73%; n=243) used methotrexate and 39% (n=127) received biological therapy. Daily prednisone in varying dosage was used in 12% (n=40). A CVD risk of <10% was found in 57% (n=188), 23% (n=77) had a CVD risk of 10-19% and 18% (n=60) had a CVD risk ≥20%. In six patients CVD risk could not be calculated due to missing data.

Table 1. General characteristics of study participants (n=328). Data are given as mean ± SD, number (%) or as median [Interquartile Range].

	RA (n=328)
Male (n,%)	104 (32%)
Age (yrs)	53 ± 11
BMI (kg/m^2)	26.5 ± 4.5
Current Smoker	60 (18%)
Systolic BP (mmHg)	132 ± 19
Diastolic BP (mmHg)	79 ± 10
Glucose (mmol/L)	5.5 ± 0.6
Total cholesterol (mmol/L)	5.4 ± 1.1
LDL-C (mmol/L)	3.4 ± 0.9
HDL-C (mmol/L)	1.48 ± 0.41
Triglycerides (mmol/L)	1.05 [0.73-1.51]
Apo AI (g/L)	1.69 ± 0.40
Apo B (g/L)	1.00 ± 0.26
Apo B48 (mg/L) (median [IQR])	8.6 [5.2-12.5]
Remnant-C (mmol/L)	0.52 ± 0.26
Medication use	
Lipid lowering drug (n,%)	15 (5%)
Antihypertensive drugs (n,%)	56 (17%)
RA disease activity (DAS28) (median [IQR])	2.30 [1.60-3.24]

CAD = coronary artery disease; BP = blood pressure; Remnant-C = total cholesterol -(LDL-C) – (HDL-C).

Correlation studies

Remnant cholesterol (remnant-C) concentration was 0.52±0.26 mmol/L and fasting plasma triglycerides 1.25±0.88 mmol/L. The median apo B48 was 8.6 [IQR 5.2-12.5] mg/L. Apo B48 correlated positively with triglycerides (r=0.651; P<0.001), remnant-C (r=0.479; P<0.001), total cholesterol (r=0.196; P< 0.001), LDL-C (r=0.123; p=0.03) and total serum apo B (r=0.261; p<0.001) (Figure 1). A negative correlation was found between apo B48 and HDL-C (r=-0.208; P<0.001). A positive trend was observed between apo B48 and glucose (r=0.106; p=0.06), and between apo B48 and systolic blood pressure (r=0.095; p=0.09). No significant correlation was found between apo B48 and BMI (r=0.014; p=0.81), waist circumference (r=0.067; p=0.23) or DAS28 (r=-0.03; p=0.58).

General characteristics based on apoB48 tertiles

Tertiles based on apo B48 levels showed that patients in the highest tertile were more often male (43% [n=46] vs. 28% [n=31]; p=0.03), were older (55±9 vs. 53±12 years; p=0.018) and more often anti-CCP positive (75% [n=62] vs. 59% [n=59]; p=0.005) and rheumatoid factor positive (75% [n=72] vs. 58% [n=62]; p=0.04) compared to the lowest

tertile. Triglycerides were higher in the highest tertile compared to the lowest tertile (median 1.49 [IQR 0.93-2.01] mmol/L vs. median 0.83 [IQR 0.65-1.09] mmol/L; p <0.001). The median [IQR] apo B48 was 4.4 [3.7-5.2] mg/L in the lowest tertile and 14.6 [12.4-18.5] mg/L in the highest tertile.

No differences were found in total cholesterol, LDL-C, HDL-C, DAS28, C-reactive protein, RA disease duration, the presence of erosive disease, DMARD use, statin use or prednisone use (data not shown).

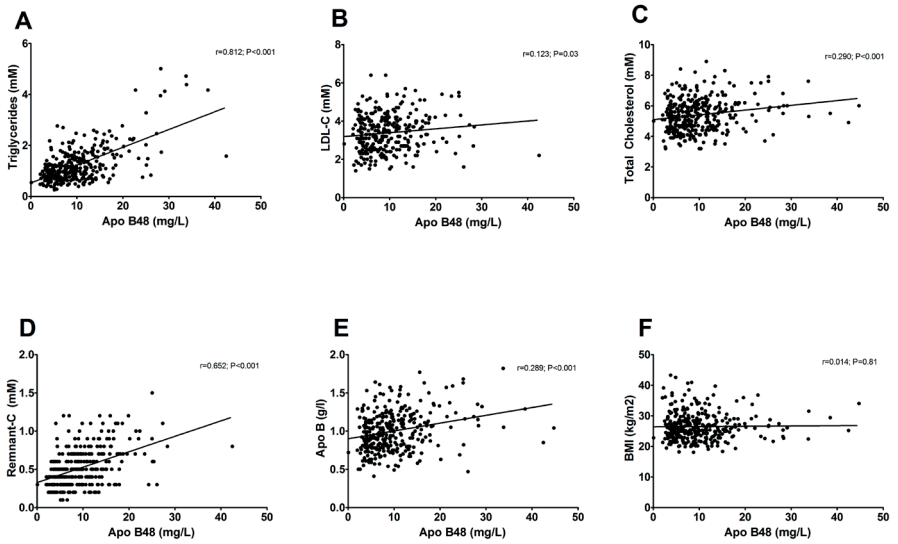

Figure 1. Correlations of apolipoprotein (apo) B48 with triglycerides (A), LDL-C (B), total cholesterol (C), remnant-cholesterol (D), apo B (E) and body mass index (F).

Discussion

This is the first study to explore apo B48 levels in RA patients, showing elevated plasma concentrations, with a median apo B48 of 8.6 [5.2-12.5] mg/L, compared to previous studies in healthy controls. Alipour et al. found apo B48 levels of 5.7±0.6 mg/L in healthy individuals and Masuda et al. found apo B48 levels of 3.9 ± 2.4 mg/L in non-CAD patients without overt coronary stenosis (26, 32). In another study, Masuda et al. investigated apo B48 levels in different subgroups of healthy controls. They concluded that apo B48 levels of 5.7 mg/L should be regarded as the upper limit of normal apo B48 levels in normolipidemic healthy controls (33). Interestingly, the highest apo B48 tertile in our RA group showed threefold increased levels compared to this suggested reference value. Although the triglyceride concentrations were higher in this tertile, those levels

were still within normal limits. Patients in the highest apo B48 tertile were significantly more often anti-CCP and rheumatoid factor positive. Anti-CCP and rheumatoid factor are associated with a more progressive disease course of the RA and thus with more inflammation. Patients in the highest tertile did not show a higher C-reactive protein, DAS28 of RA disease duration and they had not more often erosive disease. Therefore, the exaggerated apo B48 levels in this subgroup remain unexplained.

Apo B48, remnant-C and triglycerides are all markers of postprandial lipemia and represent different components of the lipoprotein fractions. Apo B48 reflects the number of circulating chylomicrons and their respective remnants, while remnant-C and plasma triglycerides reflect the lipid content within the chylomicrons and remnants, including VLDL and its remnants. Interestingly, apo B48 was increased in RA while triglycerides and remnant-C were within normal range, reflecting a high number of circulating, relatively small and probably cholesterol-depleted remnants. Therefore, in RA the lipolysis of chylomicrons may be normal, while the hepatic catabolism of chylomicron remnants may be impaired. Alternatively, the intestinal production of chylomicrons may be increased, similar to the situation in T2DM (22). Further investigation of the metabolism of triglyceride-rich lipoproteins and the role of postprandial hyperlipidemia on the excess CVD risk in RA patient is necessary. It would be interesting to know whether there is an association between apo B48 and levels of auto-antibodies. These data were unavailable in this study.

In conclusion, apo B48 levels in RA patients are high, despite low triglycerides and remnant-C, which indicates that chylomicron remnant clearance may be delayed in RA. The current data suggest an association between apo B48 levels with rheumatoid factor or anti-CCP positivity in RA.

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