Association of rheumatoid arthritis disease activity and antibodies to periodontal bacteria with serum lipoprotein profile in drug naive patients

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Running head: Association of RA disease activity and LDL

Original article

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ABSTRACT

Objective. We investigated lipid concentrations, particle sizes and antibodies binding to periodontal bacteria *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, and to malondialdehyde-acetaldehyde modified low-density lipoprotein in immunoglobulin class A, G, and M among patients with newly diagnosed rheumatoid arthritis in a population-based cohort.

Methods. Concentrations and sizes of lipoprotein particles analysed by proton nuclear magnetic resonance spectroscopy and antibody levels to malondialdehyde-acetaldehyde modified low-density lipoprotein were studied at baseline and after one-year of follow-up. Serum immunoglobulin A and G class antibodies to periodontal bacteria were determined at baseline.

Results. Sixty-three patients were divided into tertiles according to disease activity by disease activity score with 28 joint count and erythrocyte sedimentation rate (<3.9, 3.9-4.7, >4.7). Small low-density lipoprotein concentration was lowest in the tertile with the highest disease activity. In high-density lipoprotein, the concentrations of total, medium and small particles decreased with disease activity. The particle size in low-density lipoprotein associated with disease activity and the presence of antibodies to *Porphyromonas gingivalis*. Immunoglobulin G and M antibodies to malondialdehyde-acetaldehyde modified low-density lipoprotein correlated with disease activity. Inflammation associated changes faded by one year.

Conclusion. Drug naive RA patients had proatherogenic changes in lipid profiles, but they were reversible, when inflammation diminished.

Keywords: rheumatoid arthritis, LDL cholesterol, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, malondialdehyde-acetaldehyde adduct

Key messages

Patients with drug naive rheumatoid arthritis showed proatherogenic lipid profiles.

Reversible changes in lipid profiles can be achieved as response to inflammation suppression.

Active therapy aimed at remission is essential in all patients with rheumatoid arthritis.

INTRODUCTION

Patients with rheumatoid arthritis (RA) have increased cardiovascular morbidity and mortality (1). Proatherogenic lipid profile has been reported in drug naive RA patients without comorbidities (2). The role of low-density lipoproteins (LDL) is crucial in atherogenesis, and both concentration and composition of LDL influence the cardiovascular risk (3). In addition to transporting lipids throughout the body, protein compositions have an impact in thrombosis, iron transport, immune function and acute phase response (4,5). Post-translational protein modifications increase the functional diversity of the proteome, but may also cause organ dysfunction in chronic diseases (6). Modified LDL occurs in diseases characterized by increased oxidative stress (7). Lipoprotein particles can be modified in multiple ways which differ in their ability to induce fusion (8,9). Apolipoprotein B-100 can be misfolded in LDL causing an increase in β-sheet structure which primes aggregation of native LDL (10).

Carbamylation, a form of post-translational modification, can occur spontaneously or via a route assisted by myeloperoxidase (11). Myeloperoxidase catalyses the oxidation of thiocyanate to cyanate. The active form of cyanate acts as a potential toxin and interacts with the amine groups of proteins generating homocitrulline (12). Smoking elevates serum thiocyanate levels and may facilitate carbamylation by myeloperoxidase. Development of seropositive RA is associated with smoking (13). As a proof of in vivo occurrence, immunoglobulin (Ig) G antibodies recognizing homocitrulline-containing antigens in serum, carbamylated Igs in synovial fluid, and protein-bound homocitrulline in joint tissues have been described in RA (reviewed in 14). Carbamylation also occurs in lipoprotein particles. Carbamylation of 15% of lysine residues completely abolished the interaction of LDL particle with its receptor (15). Extensively carbamylated LDL is efficiently

cleared from the circulation, whereas minimally carbamylated LDL has decreased clearance (16).

In RA, serum malondialdehyde level is increased as a marker of lipid peroxidation (17), and in the presence of acetaldehyde highly immunogenic malondialdehyde-acetaldehyde (MAA) adducts are produced (18). Among RA patients with a mean disease duration of twelve years the antibody responses to MAA associated in IgA- and IgG-class both with rheumatoid factor and anti-citrullinated protein antibodies, and in IgM-class only with rheumatoid factor (18).

Bacterial infections have been suspected to be involved in lipoprotein modifications and atherothrombotic events (19,20). Neutrophils constitute the first line of defence against bacteria. Oral biofilm triggers neutrophil extracellular trap formation in which myeloperoxidase participates (21). In a population of almost 7000 subjects, IgA-seropositivity for *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) was associated with stroke incidence in subjects free from cardiovascular disease at baseline, and IgA-seropositivity for *Porphyromonas gingivalis* (*P. gingivalis*) in subjects with a history of cardiovascular disease (22). The presence of *P. gingivalis* influenced the aggregation and mobility of LDL, which also bound to specific proteins of *P. gingivalis* (23). Natural IgM antibodies recognize molecular mimicry between epitopes of oxidatively modified lipoproteins and pathogen associated molecular patterns (24). Such antibodies recognize gingipain of *P. gingivalis* which shares molecular identity with epitopes on malondialdehyde-LDL (25). *A. actinomycetemcomitans* and *P. gingivalis* are common periodontal bacteria, and their amount is strongly associated with aggressive and chronic periodontitis (26). Serum antibody levels to these bacteria are determined by their amount and by the severity of periodontitis (27). Both species may also cause systemic infections due to hematogenous dissemination from the infected periodontium, and have been associated with local infections in various parts of the body outside the oral cavity (28).

Here we report differences in the concentrations and sizes of lipoprotein subclass particles in relation to disease activity and the presence of antibodies to periodontal pathogens as well as to MAA-LDL in patients with drug naive RA at baseline and after one year of follow-up.

PATIENTS AND METHODS

Patients. Drug naive patients with RA were collected by the rheumatologists working in the Northern Savo outpatient departments (29). Patients filled in questionnaires about their symptoms, comorbidities, smoking, use of alcohol, patient's global assessment of disease activity (10 cm visual analogue scale), and assessment of physical function (Health Assessment Questionnaire). Data on age, gender and symptom duration was recorded. On the first visit, tender and swollen joint count (out of 66/68 joints) and patient's weight and waist circumference were measured. Height was self-reported. Sitting blood pressure (BP) was measured at the visit. Body mass index was calculated as weight (kg) divided by height squared (m^2). Basic laboratory tests such as erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein, rheumatoid factor, anti-citrullinated protein antibodies, fasting lipid panel for total, HDL-, LDL-cholesterol, triglycerides and fasting plasma glucose was examined. At one-year follow-up visit the patients were examined accordingly. Radiographs of hands and feet were taken and scored using Sharp van der Heijde method by an experienced radiologist (LL) (30). The patients were diagnosed based on the American College of Rheumatology/European League against Rheumatism 2010 criteria (31). Disease activity score with 28 joint count and ESR (DAS28(ESR)) was used as a clinical index in assessing inflammatory activity.

Metabolic syndrome (MetS) was defined according to the National Cholesterol Education Program's Adult Treatment Panel III definition as any three or more of the following items: central obesity with waist circumference > 102cm in men and > 88cm in women, triglyserides ≥ 1.70 mmol/l, HDL < 1.03 mmol/l in men and < 1.29 mmol/l in women, systolic BP ≥ 130mmHg, diastolic BP ≥ 85mmHg, or fasting plasma glucose ≥ 5.6mmol/l. Treatment for lipids, diabetes and blood pressure are included in the classification of MetS by definition (32).

Laboratory analyses. Lipid measurements were performed as routine laboratory tests by using automated photometric enzymatic method. Serum and plasma samples were stored at -70˚C. Serum

high sensitivity C-reactive protein was measured with particle enhanced immunoturbidimetric assay (ELISA Roche Diagnostics GmbH, Mannheim, Germany). Concentrations of IL-1Ra in serum and IL-6 in plasma were measured by ELISA with commercial reagents (R&D Systems Europe Ltd., Abingdon, UK and eBioScience Inc., San Diego, CA, USA). The inter-assay coefficients of variation and the detection limit were 3.7% and 15.6 pg/mL for IL-1Ra, and 6.4% and 0.2 pg/mL for IL-6, respectively.

Serum IgA- and IgG-class antibodies against periodontal bacteria *A. actinomycetemcomitans* and *P. gingivalis* were determined by multi-serotype ELISA (33). Coefficient of variation % were 5.1 and 5.2% for *A. actinomycetemcomitans* IgA and IgG, 4.4 and 4.5% for *P. gingivalis* IgA and IgG. Seropositive results were defined as ≥ 2 ELISA units in IgA-class and ≥ 5 ELISA units in IgG-class (33).

Measurement of antibodies to MAA-LDL

Serum IgA, IgG, and IgM antibody levels to MAA-LDL were determined using chemiluminescent immunoassay (34). Briefly, MAA-LDL was immobilized on 96-well white microtiter plates. Non-specific binding sites were blocked with 0.5% fish gelatin in 0.27 mM PBS-EDTA. Serum samples (1:100 – 1:2000) were diluted in PBS-EDTA and incubated for one hour. The bound immunoglobin was determined with appropriate alkaline-phosphatase-conjugated secondary antibodies and Lumi-Phos (Lumigen, MI) as substrate. Data are expressed as relative units determined from internal human immunoglobulin standard-curve.

Lipoprotein subclass analysis by proton nuclear magnetic resonance spectroscopy

Concentrations of lipoprotein subclasses were analysed by proton nuclear magnetic resonance spectroscopy of native serum samples (35). Serum samples stored in -70˚C were thawed overnight in a refrigerator and analysed in a single batch. The proton nuclear magnetic resonance data were measured at 37˚C using a Bruker AVANCE III spectrometer operating at 500 MHz using an

automated platform which has been described in detail previously (35). The lipoprotein subclasses were calibrated using high-performance liquid chromatography and defined according to the following criteria: 1) as one of six VLDL subclasses extremely large (with particle diameters from approximately 75 nm upwards), very large (average particle diameter of 64.0 nm), large (53.6 nm), medium (44.5 nm), small (36.8 nm), and very small (31.3 nm); 2) as IDL (28.6 nm); 3) as one of three LDL subclasses large (25.5 nm), medium (23.0 nm), and small (18.7 nm); and 4) as one of four HDL subclasses very large (14.3 nm), large (12.1 nm), medium (10.9 nm), and small (8.7 nm). In our analyses, "large" VLDL particles included extremely large, very large and large VLDL particles and "small" VLDL particles included small and very small VLDL particles. IDL particles and large LDL particles were combined as "large" LDL particles, and, respectively, very large and large HDL particles were combined as "large" HDL particles. Hence, three subclasses (large, medium and small) for VLDL, LDL and HDL particles were used in analysing the subclass concentrations. The mean size of the VLDL, LDL, and HDL particles was calculated by weighing the corresponding subclass diameters with their particle concentrations.

Statistics.

The subjects were divided into tertiles according to DAS28(ESR) (<3.9, 3.9-4.7, >4.7). The data are presented as means with standard deviations (SD) or counts with percentages. We used the t-test or paired type t-test analyses to compare continuous variables between two groups. The mean changes between the outcome variables are presented with 95% confidence intervals (CI). Generalized linear models with appropriate distribution and link function was used in comparison between groups and for the hypotheses of linearity evaluation. In the case of violation of the assumptions (e.g. non-normality), a bootstrap-type test was used. The normality of the variables was tested by using the Shapiro-Wilk W test. We calculated by the Pearson method the correlation coefficients for linear dependency. The 95% CI were obtained by bias-corrected, accelerated bootstrapping (3000 replications). Spearman correlation was calculated while assessing relationship between two

variables. STATA 13.1, StataCorp LP (College Station, TX, USA) statistical package was used for the analyses.

Ethics. The study was approved by the Ethics Committee of the Kuopio University Hospital. All patients gave written consent.

RESULTS

Demographic, clinical and laboratory data on 63 patients, 34 females and 29 males, based on disease activity by DAS28 (ESR) in tertiles <3.9, 3.9-4.7, >4.7 are shown in Table 1. Of the participants, 27% were on lipid lowering, 38% on antihypertensive, and 10% on antihyperglycemic medication. One fourth were current smokers, and 68% reported ever use of alcohol. Ninety-two percent of patients had either IgA or IgG antibodies to *A. actinomycetemcomitans*, and 61% had either IgA or IgG antibodies to *P. gingivalis*.

Total and subclass cholesterol lipoprotein particle concentrations and their mean sizes in RA disease activity tertiles are shown in Table 2. In LDL subclass, small particle concentration, and in HDL subclass, the total, medium and small particle concentrations decreased significantly with disease activity. The linear decreases in total LDL and HDL particle concentrations are shown in Figure 1. The LDL particle diameters associated with disease activity as the particle sizes increased with increasing DAS28(ESR) as shown in Table 2 and Figure 1. The presence of IgA antibodies to *A. actinomycetemcomitans* had no influence on the mean diameters of LDL particles as the mean difference between the groups was 0.09 nm (95% CI; 0.085 to 0.27, p=0.28), whereas in the presence of IgA antibodies to *P. gingivalis* the diameters of LDL particles increased, 23.7±0.2 vs 23.6±0.2 nm, with a mean difference of 0.14 nm (95% CI; 0.04 to 0.24, p=0.007). The mean diameter of LDL particles in patients with or without anticitrullinated protein antibodies did not differ significantly, as the mean difference between the groups was 0.05 nm (95% CI; -0.01 to 0.21, p=0.074). Serum IgG and IgM antibody levels to MAA-LDL showed moderate association with

disease activity as shown in Figure 2.

All patients were on anti-rheumatic therapy with disease-modifying antirheumatic drugs, two-thirds mainly with a combination of two or three of the following drugs: methotrexate, hydroxychloroquine and sulphasalazine, and half were also on low-dose prednisolone. One-year follow-up data was available for 54 persons. At one year the therapy was continued mainly with the combinations of the aforementioned disease-modifying antirheumatic drugs and a third of the patients were still on low-dose prednisolone. Two patients were on azathioprine and one on sodiumaurothiomalate. One patient was also on anakinra due to systemic features of the disease and one was treated with rituximab during the first year. The benefit of therapy was shown, as the mean DAS28(ESR) (SD) decreased from 4.3(1.3) to 2.0 (1.2) with a mean difference of 2.3 (95% CI; 1.89 to 2.69, $p<0.001$), between the baseline and follow-up visits. DAS28(ESR) at baseline showed a weak association with an increase in LDL concentration between baseline and one-year follow-up, whereas the increase was more significant in HDL concentration and in anti-MAA-IgM level as shown in Table 3. The mean diameter of LDL particles decreased significantly. Decrease in anti-MAA-LDL-IgM level showed a moderate positive association with a combination therapy of disease-modifying antirheumatic drugs (n=29, r=0.40, 95% CI; 0.15 to 0.60, p=0.003), and a weak positive association with the use of prednisolone (n=19, r=0.30, 95% CI; 0.03 to 0.53, p=0.024), or sulphasalazine (n=28, r=0.33, 95% CI; 0.06 to 0.55, p=0.011) as a part of medication, but not with the use of hydroxychloroquine (n=31) or methotrexate (n=31).

DISCUSSION

The present study showed marked changes in LDL and HDL concentrations typical of chronic inflammation and its proatherogenic profile (2,36). They were inversely proportional to the degree of inflammatory changes. An increase in LDL particle size associated with disease activity and the presence of antibodies binding to *P. gingivalis*. LDL particle diameters were larger in patients with *P. gingivalis* antibodies present than absent. The largest differences were observed in the highest

disease activity tertile in which also antibodies to periodontal bacteria were most frequently recorded. Anti-MAA-LDL in IgG and IgM class increased with disease activity and the greatest increase in IgM occurred in patients with the highest DAS28(ESR) at baseline.

In an earlier study, in which patients had treated, but active RA with a mean disease duration of ten years and no serious comorbidities, RA patients had significantly higher levels of small, dense LDL and lower levels of large, light LDL than the controls (37). LDL particle size was smaller in RA patients compared with the controls, 20.9 vs 21.2, whereas HDL particle size was greater than in the controls due to a decrease in the concentration of small dense HDL particles (37). The mean LDL particle sizes in both groups were lower than among the patients in our series. Opposite to the results of the present study, a study from Turkey reported that drug naive patients with early RA without comorbidities had a strong reduction of large LDL particles with a concomitant increase in the smallest, most dense LDL (38). This led to a reduced LDL particle size. Forty percent of the patients had elevated levels of small, dense LDL compared with the healthy controls. In that study, subjects with any chronic comorbidity or therapy with drugs known to affect lipid metabolism were excluded (38). The mean LDL particle size was greater than in the present study, 26.4 vs 23.7 nm. The diversity observed in the LDL particle size between the studies most probably reflects patient selection concerning comorbidities, disease duration and therapy (37,38).

Serum amyloid A (SAA), an inflammatory mediator in RA, stimulates the synthesis of vascular proteoglycans which are known to bind LDL with high affinity, thereby contributing to increased lipoprotein retention in subendothelial space (39). In the presence of electronegative LDL(-) with misfolded apolipoproteins LDL can undergo amyloidogenic aggregation (10). Therefore, conformational changes in apolipoprotein B-100 may influence particle size (9,10). SAA has also been shown to increase the particle size of lipoproteins (40).

In 1997 in randomly selected adults aged 25-54 years, as a part of Finnish North Karelian and Russian Karelian study, the prevalence of IgG antibodies to *A. actinomycetemcomitans* was 40.9 %

and IgG antibodies to *P. gingivalis* 28.3 % in Finland (41). The prevalence of these antibodies in the present study was more than two-fold higher. However, our patients had RA and were significantly older. The prevalence of antibodies to these bacteria and other common infectious agents was much higher in Russian Karelian population probably protecting them from atopy. In the Finnish North Karelian and Russian Karelian population cohorts, the patients with the highest infection burden measured as elevated antibody levels to a group of pathogens (*A. actinomycetemcomitans*, *P. gingivalis* and herpes simplex virus) had the lowest HDL cholesterol concentrations (42). In another study, in male subjects antibody levels to *A. actinomycetemcomitans* associated with low HDL cholesterol concentration and MetS (43). Periodontitis was also shown to change HDL composition, which impaired its efflux capacity (44). Such changes diminish antiatherogenic potency of HDL in a similar way to an acute-phase response.

In an animal study, *A. actinomycetemcomitans* challenge promoted oxidation of LDL, probably contributing to inflammation and atherosclerosis (45). In a different animal model degradation of apolipoprotein B-100 by *P. gingivalis* gingipain R played a crucial role in the development of atherosclerosis (46). The effect of *P. gingivalis* to LDL composition was also tested in laboratory conditions (47). When HDL and LDL particles prepared from whole blood were stimulated by *P. gingivalis*, LDL was proteolyzed into distinct peptide fragments, and LDL particles became oxidatively modified. *P. gingivalis* modified LDL had increased the amount of apolipoprotein M (47). LDL modification resulted in aggregated lipid particles that can be taken up by macrophages to form foam cells, hallmarks of early atherosclerosis (17,45,46). Modifications in LDL structure also influence its binding to the receptor (10,11,47-49). The selectively modified arginyl residues of human LDL almost totally abolished the binding of LDL to the high affinity cell surface receptors of human fibroblasts (49). In the present study, reversible changes in LDL particle diameters with diminished inflammation support early active therapy in RA to prevent permanent modifications in LDL particle structure.

The limitations of the present study are a small sample size and the lack of a population-based non-arthritis control group. Data was prospectively collected as a part of an epidemiological survey during one year (41). The study design did not include the controls. All incident cases were not willing or able to participate in a more thorough protocol with filling questionnaires and giving extra blood samples. Conclusions on anti-citrullinated protein antibodies positive and negative subjects were also limited by the small sample size. However, in a population-based survey to monitor the health of the Finnish population among persons aged 25 to 64 at recruitment in 1997 (Finrisk study) the mean LDL diameter was 23 nm, which was below the lowest tertile of the present study (50,51). In the Finrisk study the subjects were younger (mean age 48 vs 59 years), used less often lipid lowering medication (2.6 vs 15.9 %), and had less diabetes (5.4 vs 9.5%) compared to the present study; these facts may explain the different results in these studies. The mean BMI and the number of current smokers were comparable.

The strength of the present study is that it was population based, and all drug naive patients were included regardless of comorbidities. In the aforementioned Turkish study the mean DAS28 was 6.2, higher than disease activity in the highest tertile in our study (38). Opposite to the Turkish study, the concentration of small LDL particles decreased with disease activity, and the particle size increased, which may be due to different patient selection, e.g. lower disease activity and treated comorbidities, or thorough analysis of the result in relation to disease activity. Corresponding to an earlier study on patients with active, established RA, small LDL and total, medium and small HDL particle concentrations were lowest in the tertile with the highest disease activity (37). In hypercholesterolemic patients, atorvastatin therapy increased both LDL and HDL particle size and HDL particle concentration (52). In our study, the patients in the highest disease activity tertile were twice more probable users of lipid lowering medication compared to the patients in the lowest tertile. In the highest tertile the concentration of small LDL particles was also lowest influencing the mean particle size. Increase in LDL particle size is regarded as a beneficial effect in treating

hypercholesterolemia, whereas in inflammation it may be an opposite sign due to a quicker retention of the smallest particles into endothelium. In our study the concentrations of medium and small HDL particles decreased with inflammation suggesting that current lipid lowering therapy was not able to overcome the effect of inflammation.

In the present study IgG- and IgM-anti-MAA-LDL correlated with RA disease activity. In a study from Sweden, IgG and IgM antibodies to malondialdehyde-LDL were increased in patients with RA and especially, increased prevalence of IgG antibodies was associated with myocardial infarction (53). In a US study, both IgM- and IgG-class antibodies to MAA-LDL were associated with acute and IgA-class antibodies with chronic coronary artery disease (54). Of disease-modifying antirheumatic drugs, methotrexate has shown to reduce MAA formation by inhibiting activation of redox signaling pathways (55). Natural IgM bind to epitopes produced by oxidative stress (24). In our study, the RA patients were drug-naive with mean symptom duration less than one year which may have influenced the antibody spectrum. Baseline disease activity associated with a change in IgM antibody level to MAA-LDL between baseline and one-year follow-up, whereas in other Ig classes no significant changes were recorded. The IgM antibody level to MAA-LDL decreased which is a beneficial effect considering cardiovascular risks (54). We could not show any beneficial effect of methotrexate in this study probably due to the small number of cases. However, association of decreased IgM antibody level to MAA-LDL and use of combinations of disease-modifying antirheumatic drugs, or use of prednisolone or sulphasalazine as a part of the medication was recorded, which most probably resulted from inflammation suppression. Improved HDL function and increase in LDL have associated with a decrease in disease activity in several studies on both synthetic and biological drugs (36).

Changes in the structure of particles, aggregated particles and carrier function for acute phase proteins together may explain the increase in the mean LDL particle size with disease activity at diagnosis. Although decreases in the concentrations of medium and small HDL and small LDL

were recorded, the particle size increased only in LDL. In this series antibodies to *P. gingivalis* may also have an effect on the increase of LDL particle sizes, although no causal relationship can be verified in this type of observational study. Reversible changes in lipid profiles as response to inflammation suppression support active therapy for newly diagnosed patients with RA.

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REFERENCES

1. van Doornum S, McColl G, Wicks IP. Accelerated Atherosclerosis: An extra-articular feature of rheumatoid arthritis? Arthritis Rheum. 2002;46:862–73.
2. Georgiadis AN, Papavasiliou EC, Lourida ES, et al. Atherogenic lipid profile is a feature of patients with early rheumatoid arthritis: effect of early treatment–a prospective, controlled study. Arthritis Res Ther. 2006;8:R82.
3. Hörkkö S, Binder CJ, Shaw PX, et al. Immunological responses to oxidized LDL. Free Radic Biol Med. 2000; 28: 1771–9.
4. Dashty M, Motazacker MM, Levels J, et al. Proteome of human plasma very low-density lipoprotein and low-density lipoprotein exhibits a link with coagulation and lipid metabolism. Thromb Haemost. 2014;111:518-30.
5. Shah AS, Tan L, Long JL, et al. Proteomic diversity of high density lipoproteins: our emerging understanding of its importance in lipid transport and beyond. J Lipid Res. 2013;54:2575-85.
6. Gillery P, Jaisson S. Post-translational modification derived products (PTMDPs): toxins in chronic disease? Clin Chem Lab Med. 2014;52:33-8.
7. Pentikäinen MO, Lehtonen EMP, Kovanen PT. Aggregation and fusion of modified

low density lipoprotein. J Lipid Res. 1996;37:2638-49.

8. Diffenderfer MR, Schaefer EJ. The composition and metabolism of large and small LDL. Curr Opin Lipidol. 2014;25:221-6.
9. Bancells C, Villegas S, Blanco FJ, et al. Aggregated electronegative low density lipoprotein in human plasma shows a high tendency toward phospholipolysis and particle fusion. J Biol Chem. 2010;285:32425–35.
10. Parasassi T, De Spirito M, Mei G, et al. Low density lipoprotein misfolding and amyloidogenesis. FASEB J. 2008;22:2350–6.
11. Wang Z, Nicholls SJ, Rodrigues ER, et al. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. Nat Med. 2007;13:1176-84.
12. Kraus LM, Kraus AP Jr. Carbamylation of amino acids and proteins in uremia. Kidney Int Suppl 2001;78:S102–7.
13. Klareskog L, Stolt P, Lundberg K, et al, and the Epidemiological Investigation of Rheumatoid Arthritis Study Group. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA–DR (shared epitope)–restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum. 2006;54:38–46.
14. Häyrynen J, Kärkkäinen M, Kononoff A, et al. Automated immunoassays for the autoantibodies to carbamylated or citrullinated telopeptides of type I and II collagens. Clin Chem Lab Med. 2014;53:1375-80. doi: 10.1515/cclm-2014-0683.
15. Gonen B, Cole T, Hahm KS. The interaction of carbamylated low-density lipoprotein with cultured cells – Studies with human fibroblasts, rat peritoneal macrophages and human monocyte-derived macrophages. Biochim Biophys Acta 1983;754:201-7.
16. Hörkkö S, Huttunen K, Kervinen K, et al. Decreased clearance of uremic and mildly carbamylated low-density lipoprotein. Eur J Clin Investig. 1994;24:105-13.
17. Ozkan Y, Yardym-Akaydyn S, Sepici A, et al. Oxidative status in rheumatoid arthritis. Clin Rheumatol. 2007;26:64–8.
18. Thiele GM, Duryee MJ, Anderson DR, et al. Malondialdehyde-acetaldehyde adducts and anti–malondialdehyde-acetaldehyde antibodies in rheumatoid arthritis. Arthritis Rheum. 2015;67:645–55.
19. Lakio L, Lehto M, Tuomainen AM, et al. Pro-atherogenic properties of lipopolysaccharide from the periodontal pathogen Actinobacillus actinomycetemcomitans. J Endotoxin Res. 2006;12:57-64.
20. Qi M, Miyakawa H, Kuramitsu HK. Porphyromonas gingivalis induces murine macrophage foam cell formation. Microbiol Pathogenesis 2003;35:259-67.

21. Hirschfeld J, Dommisch H, Skora P, et al. Neutrophil extracellular trap formation in supragingival biofilms. Int J Med Microbiol. 2015;305:453-63.
22. Pussinen P, Alfthan G, Rissanen H, et al. Antibodies to periodontal pathogens and stroke risk. Stroke 2004;35:2020-3.
23. Miyakawa H, Honma K, Qi M, et al. Interaction of Porphyromonas gingivalis with low-density lipoproteins: implications for a role for periodontitis in atherosclerosis. J Periodont Res. 2004;39:1–9.
24. Binder CJ. Natural IgM antibodies against oxidation-specific epitopes. J Clin Immunol. 2010;30 Suppl 1: S56–60.
25. Turunen SP, Kummu O, Harila K, et al. Recognition of Porphyromonas gingivalis gingipain epitopes by natural IgM binding to malondialdehyde modified low-density lipoprotein. PlosOne 2012;7:e34910
26. Rylev M, Kilian M. Prevalence and distribution of principal periodontal pathogens worldwide. J Clin Periodontol. 2008;35(8 Suppl):346-61.
27. Pussinen PJ, Könönen E, Paju S, et al. Periodontal pathogen carriage, rather than periodontitis, determines the serum antibody levels. J Clin Periodontol. 201138:405-11.
28. Parahitiyawa NB, Jin LJ, Leung WK, et al. Microbiology of odontogenic bacteremia: beyond endocarditis. Clin Microbiol Rev. 2009;22:46–64.
29. Kononoff A, Arstila L, Pussinen P, et al. Incidence of inflammatory joint diseases in Finland - Results from a population-based epidemiological study. Rheumatol Int. 2017;37:1693–700. DOI: 10.1007/s00296-017-3779-1
30. van der Heijde D. How to read radiographs according to the Sharp/van der Heijde method. J Rheumatol. 2000;27:261-3.
31. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2010;69:1580-8.
32. Grundy S, Brewer HB, Cleeman JI, et al. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004;109:433-8.
33. Pussinen PJ, Vilkuna-Rautiainen T, Alfthan G, et al. Multiserotype enzyme-linked immunosorbent assay as a diagnostic aid for periodontitis in large-scale studies. J Clin Microbiol. 2002;40:512-8.

34. Vehkala L, Ukkola O, Kesäniemi YA, et al. Plasma IgA antibody levels to malondialdehyde acetaldehyde-adducts are associated with inflammatory mediators, obesity and type 2 diabetes. Ann Med. 2013;45:501-10.
35. Soininen P, Kangas AJ, Würtz P, et al. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. Analyst 2009;134:1781-5.
36. Myasoevadova E. Lipids and lipid changes with synthetic and biologic disease-modifying antirheumatic drug therapy in rheumatoid arthritis: implications for cardiovascular risk. Curr Opinion Rheumatol. 2017;29:277-84.
37. Hurt-Camejo E, Paredes S, Masana L, et al. Elevated levels of small, low-density lipoprotein with high affinity for arterial matrix components in patients with rheumatoid arthritis: Possible contribution of phospholipase A2 to this atherogenic profile. Arthritis Rheum. 2001;44:2761-7.
38. Rizzo M, Spinas GA, Cesur M, et al. Atherogenic lipoprotein phenotype and LDL size and subclasses in drug-naive patients with early rheumatoid arthritis. Atherosclerosis 2009;207:502–6.
39. Sartipy P, Camejo G, Svensson L, et al. Phospholipase A2-modification of low density lipoproteins forms small, high density particles with increased affinity for proteoglycans and glycosaminoglycans. J Biol Chem. 1999;274:25913–20.
40. King VL, Thompson J, Tannock LR. Serum amyloid A in atherosclerosis. Curr Opin Lipidol. 2011;22:302–7.
41. von Hertzen LC, Laatikainen T, Mäkelä MJ, et al. Infectious burden as a determinant of atopy - a comparison between adults in Finnish and Russian Karelia. Int Arch Allergy Imm. 2006;140;89-95.
42. Vilkuna-Rautiainen T, Pussinen PJ, Roivainen M, et al. Serum antibody response to periodontal pathogens and herpes simplex virus in relation to classic risk factors of cardiovascular disease. Int J Epidemiol 2006;35:1486–94.
43. Hyvärinen K, Salminen A, Salomaa V, Pussinen PJ. Systemic exposure to a common periodontal pathogen and missing teeth are associated with metabolic syndrome. Acta Diabetol. 2015;52:179-82.
44. Pussinen PJ, Jauhiainen M, Vilkuna-Rautiainen T et al. Periodontitis decreases the antiatherogenic potency of high density lipoprotein. J Lipid Res 2004;45:139–47.
45. Jia R, Kurita-Ochiai T, Oguchi S, et al. Periodontal pathogen accelerates lipid peroxidation and atherosclerosis. J Dent Res. 2013;92:247-52.

46. Hashimoto M, Kadowaki T, Tsukuba T, et al. Selective proteolysis of apolipoprotein B-100 by Arg-gingipain mediates atherosclerosis progression accelerated by bacterial exposure. J Biochem. (Tokyo) 2006;140:713–23.
47. Bengtsson T, Karlsson H, Gunnarsson P, et al. The periodontal pathogen Porphyromonas gingivalis cleaves apolipoprotein B-100 and increases the expression of apoM in LDL in whole blood leading to cell proliferation. J Intern Med. 2008;263:558–71.
48. Weisgraber KH, Innerarity TL, Mahley RW. Role of lysine residues of plasma lipoproteins in high affinity binding to cell surface receptors on human fibroblasts. Biol Chem. 1978;253:9053-62.
49. Mahley RW, Innerarity TL, Pitas RE, et al. Inhibition of lipoprotein binding to cell surface receptors of fibroblasts following selective modification of arginyl residues in arginine-rich and B apoproteins. J Biol Chem. 1977;252:7279-87.
50. Borodulin K, Vartiainen E, Peltonen M, et al. Forty-year trends in cardiovascular risk factors in Finland. Eur J Public Health 2015;25:539-46. doi: 10.1093/eurpub/cku174
51. Würtz P, Havulinna AS, Soininen P, et al. Metabolite profiling and cardiovascular event risk. A prospective study of 3 population-based cohorts. Circulation 2015;131:774-85.
52. Ikewaki K, Terao Y, Ozasa H, et al. Effects of atorvastatin on nuclear magnetic resonance-defined lipoprotein subclasses and inflammatory markers in patients with hypercholesterolemia. J Atheroscler Thromb. 2009;16:51-6.
53. Cvetkovic JT, Wållberg-Jonsson S, Ahmed E, et al. Increased levels of autoantibodies against copper-oxidized low density lipoprotein, malondialdehyde-modified low density lipoprotein and cardiolipin in patients with rheumatoid arthritis. Rheumatology 2002;41:988–995.
54. Anderson DR, Duryee MJ, Shurmur SW, et al. Unique antibody responses to malondialdehyde-acetaldehyde (MAA)-protein adducts predict coronary artery disease. PloSone 2014;9:e107440.
55. Zimmerman MC, Clemens DL, Duryee MJ, et al. Direct antioxidant properties of methotrexate: Inhibition of malondialdehyde-acetaldehyde-protein adduct formation and superoxide scavenging. Redox Biol. 2017;13:588-93.

Table 1. Demographic, clinical and laboratory data on patients with untreated rheumatoid arthritis according to disease activity measured by DAS28(ESR) by tertiles.

	DAS28(ESR) tertiles		
	I (<3.9) n =21	II (3.9 – 4.7) n =21	III (>4.7) n =21
Female, n (%)	11 (52)	14 (67)	9 (43)
Age, years, (SD)	56 (12)	58 (14)	63 (10)
RF positive, n (%)	18 (86)	14 (70)	14 (67)
ACPA positive, n (%)	18 (86)	16 (76)	10 (48)
Symptom duration, months, (SD)	13 (11)	11 (11)	7 (5)
ESR, mm/h, (SD)	7.5 (5.4)	22.1 (16.3)	41.0 (22.3)
hs-CRP, mg/L, (SD)	3.1 (2.6)	18.5 (44.8)	54.5 (55.3)
Interleukin-1Ra, pg/mL, (SD)	410 (109)	697 (648)	528 (162)
Interleukin-6, pg/mL, (SD)	9.1 (5.2)	14.2 (9.8)	18.4 (22.8)
Glycoprotein acetyls, mmol/L, (SD)	1.4 (0.2)	1.6 (0.3)	1.8 (0.3)
Aa-IgA, EU, (SD)	4.8 (2.4)	5.3 (3.7)	6.4 (4.0)
Pg-IgA, EU, (SD)	3.6 (3.2)	3.1 (3.4)	8.1 (8.0)
HAQ, (SD)	0.3 (0.3)	0.6 (0.5)	1.2 (0.8)
Radiographic changes, SvdH, (SD)	6.0 (10.3)	1.2 (3.1)	0.6 (2.1)
Waist, cm, (SD)			
Men	97 (12)	95 (16)	102 (13)
Women	92 (12)	91 (12)	88 (14)
Body mass index, kg/m², (SD)	27.9 (6.3)	27.4 (5.7)	26.7 (4.2)
MetS, (%)	6 (29)	8 (38)	12 (57)
Total cholesterol, mmol/L, (SD)	5.17 (0.96)	4.90 (1.04)	4.58 (0.88)

HDL cholesterol, mmol/L, (SD)	1.68 (0.33)	1.44 (0.43)	1.39 (0.48)
LDL cholesterol, mmol/L, (SD)	2.94 (0.87)	2.67 (0.97)	2.63 (0.70)
Triglycerides, mmol/L, (SD)	1.18 (0.44)	1.41 (0.59)	1.08 (0.43)
Glucose, mmol/L, (SD)	5.64 (0.69)	6.05 (1.01)	6.46 (2.02)
Medication			
Lipid lowering medication, n (%)	4 (19)	5 (24)	8 (38)
Antihypertensive medication, n (%)	7 (33)	9 (43)	8 (38)
Antihyperglycemic medication, n (%)	0 (0)	4 (19)	2 (10)
Current smoking, n (%)	8 (38)	2 (10)	6 (29)
Reported use of alcohol, n (%)	15 (71)	16 (76)	12 (57)

RF= rheumatoid factor, ACPA= anti-citrullinated protein antibodies, hs-CRP= high sensitivity C-reactive protein, Ra= receptor antagonist, Glycoprotein acetyls= mainly a1-acid glycoprotein, Aa-IgA= Immunoglobulin class A antibodies against Aggregatibacter actinomycetemcomitans, Pg-IgA= Immunoglobulin class A antibodies against Porphyromonas gingivalis, EU= Elisa unit, HAQ= Health assessment questionnaire, SvdH= Sharp-van der Heijde method, MetS= Metabolic syndrome defined by National Cholesterol Education Program's Adult Treatment Panel III.

Table 2. Lipoprotein particle concentrations and mean sizes among patients with RA divided into tertiles according to disease activity measured by DAS28(ESR).

	All patients n=63	DAS28(ESR) tertiles I (< 3.9) n=21	II (3.9-4.7) n=21	III (>4.7) n=21	p - value (linearity*)
Particle concentration, mean (SD)					
Total VLDL, nmol/L	82.5 (25.7)	78.7 (17.5)	90.0 (32.3)	78.7 (25.7)	0.93
Large VLDL	4.2 (3.4)	4.5 (3.1)	4.8 (3.9)	3.4 (3.0)	0.34
Medium VLDL	13.9 (7.2)	14.2 (6.0)	15.0 (8.4)	12.4 (6.9)	0.44
Small VLDL	64.4 (17.6)	60.0 (11.7)	70.1 (22.2)	63.1 (16.5)	0.68
Total LDL, nmol/L	554.4 (155.5)	567.6 (153.0)	599.8 (176.2)	495.7 (120.4)	0.16
Large LDL	271.2 (68.9)	272.3 (71.0)	289.5 (76.5)	251.8 (55.4)	0.40
Medium LDL	132.1 (40.4)	136.2 (39.6)	144.4 (46.0)	115.6 (30.2)	0.11
Small LDL	151.1 (47.8)	159.1 (43.3)	166.0 (54.3)	128.3 (37.5)	**0.031**
Total HDL, µmol/L	8.7 (1.4)	9.2 (0.9)	9.1 (1.5)	7.9 (1.4)	**0.0012**
Large HDL	1.6 (0.6)	1.6 (0.5)	1.6 (0.6)	1.5 (0.8)	0.33
Medium HDL	2.1 (0.5)	2.3 (0.4)	2.1 (0.6)	1.9 (0.4)	**0.0079**
Small HDL	5.0 (0.7)	5.3 (0.5)	5.2 (0.7)	4.6 (0.6)	**<0.001**
Mean size, nm					
VLDL	36.3 (1.3)	36.7 (1.4)	36.4 (1.2)	35.9 (1.2)	0.17
LDL	23.7 (0.2)	23.6 (0.1)	23.6 (0.2)	23.8 (0.2)	**<0.001**
HDL	9.9 (0.2)	9.9 (0.2)	9.9 (0.2)	9.9 (0.3)	0.67

*Adjusted for gender, age, body mass index, diabetes and lipid lowering medication

Table 3. Association of RA disease activity measured by DAS28 (ESR) at baseline with the mean changes in concentrations of VLDL, LDL and HDL, and diameters of VLDL, LDL and HDL particles, and IgA, IgG or IgM antibody levels to MAA-LDL between baseline and one-year follow-up.

Change	DAS28(ESR) at baseline r (95% CI)	p
Concentration of lipid		
VLDL, nmol/L	0.05 (-0.21 to 0.31)	0.70
LDL, nmol/L	0.28 (0.02 to 0.50)	0.039
HDL, µmol/L	0.38 (0.12 to 0.58)	0.004
Diameter of lipid particle, nm		
VLDL	0.09 (-0.18 to 0.35)	0.50
LDL	-0.37 (-0.57 to -0.12)	0.005
HDL	0.10 (-0.17 to 0.36)	0.45
Concentration of antibody, RU		
Anti- MAA-LDL-IgA	0.19 (-0.08 to 0.44)	0.17
Anti-MAA-LDL-IgG	0.17(-0.11 to 0.42)	0.24
Anti-MAA-LDL-IgM	0.36 (0.095 to 0.57)	0.009

MAA-LDL=malondialdehyde-adduct low density lipoprotein, RU=relative unit determined from internal human Ig standard

Figure 1. Associations between RA disease activity measured as DAS28(ESR), plasma lipoprotein concentrations and lipoprotein particle sizes for VLDL, LDL and HDL. The lines show estimated linear regression with 95% confidence intervals. DAS = disease activity score

Figure 2. Associations between RA disease activity measured as DAS28(ESR) and serum IgA, IgG, and IgM antibody levels to MAA-LDL expressed as relative units determined from internal human immunoglobulin standard-curve. The lines show estimated linear regression with 95% confidence intervals. MAA-LDL = malondialdehyde-acetaldehyde modified low density lipoprotein; RU = relative unit; DAS = disease activity score

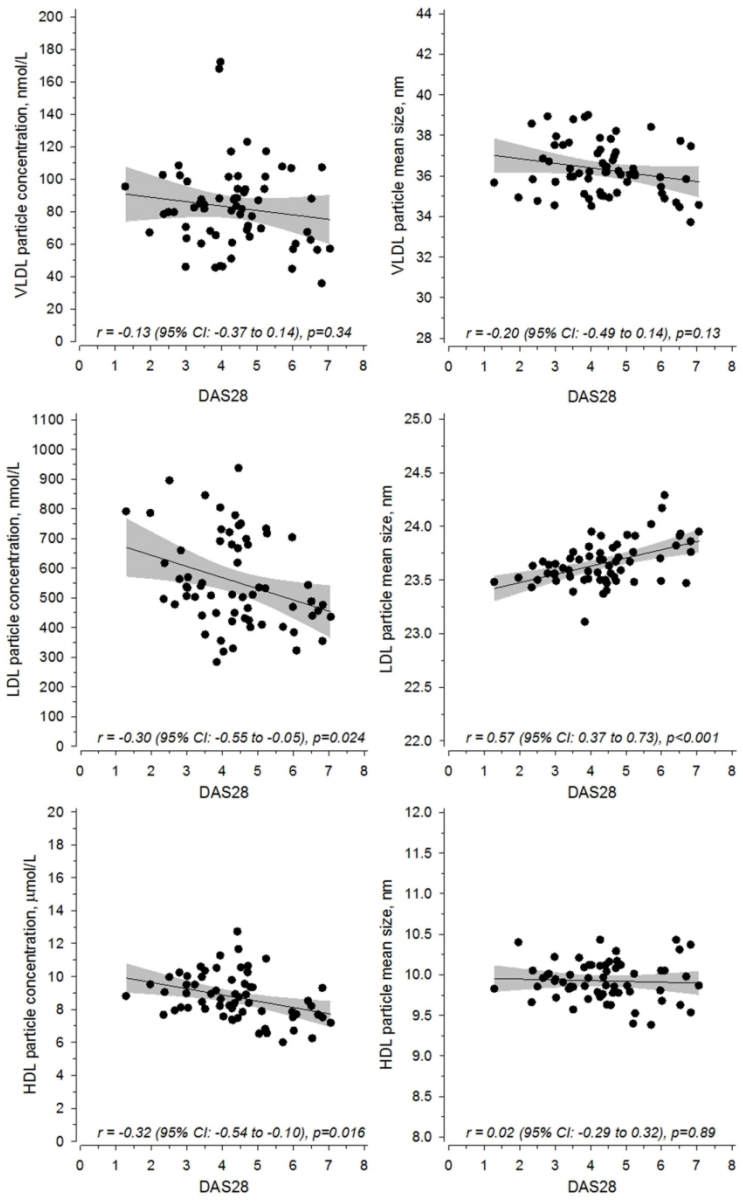

Figure 1. Associations between RA disease activity measured as DAS28(ESR), plasma lipoprotein concentrations and lipoprotein particle sizes for VLDL, LDL and HDL. The lines show estimated linear regression with 95% confidence intervals. DAS = disease activity score

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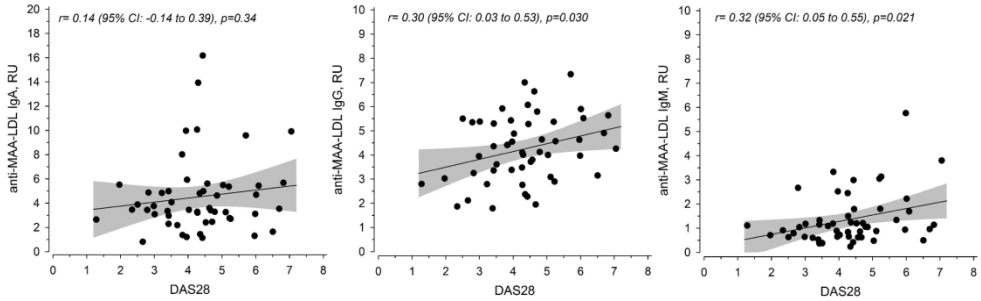

Figure 2. Associations between RA disease activity measured as DAS28(ESR) and serum IgA, IgG, and IgM antibody levels to MAA-LDL expressed as relative units determined from internal human immunoglobulin standard-curve. The lines show estimated linear regression with 95% confidence intervals. MAA-LDL = malondialdehyde-acetaldehyde modified low density lipoprotein; RU = relative unit; DAS = disease activity score

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