Antibodies to malondialdehyde-acetaldehyde modified low-density lipoprotein in patients with newly diagnosed inflammatory joint disease

Journal:	*Scandinavian Journal of Rheumatology*
Manuscript ID	SJR-2020-0053.R2
Manuscript Type:	Brief Communication
Date Submitted by the Author:	n/a
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Keywords:	Arthritis, Rheumatoid arthritis, Ankylosing spondylitis, Psoriatic arthritis, Bacterial antibodies, Antibodies

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FUNDING

This study was supported by a grant from the Rheumatism Research Foundation, the Finnish Rheumatism Association, the Finnish Cultural Foundation, Northern Savo Regional fund, and the Research Committee of the Kuopio University Hospital Catchment Area for the State Research Funding (AK, OK-S), and Sigrid Juselius Foundation and the Finnish Foundation for Cardiovascular Research (SH).

ABSTRACT

Objective. To assess antibodies to malondialdehyde-acetaldehyde modified low-density lipoprotein (MAA-LDL) in patients with newly diagnosed inflammatory joint disease.

Methods. Patients with rheumatoid arthritis (RA), spondyloarthritis (SpA), and undifferentiated arthritis (UA), participating in the Northern Savo 2010 Study were evaluated for metabolic syndrome (MetS), metabolic and inflammatory markers, antibodies to MAA-LDL, *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.

Results. Among 135 newly diagnosed untreated patients, of which 53 (39%) were diagnosed to have RA, 44 (33%) SpA, and 38 (28%) UA, 49%, 30%, and 47%, respectively, had MetS. After adjusting for age and gender, anti-MAA-LDL IgA (p=0.009), IgG (p=0.031) and IgM (p=0.001) levels differed between the diagnostic categories, but not in patients with MetS present or absent. All antibody classes to MAA-LDL correlated with erythrocyte sedimentation rate (ESR), and IgA and IgG antibodies with high sensitivity C-reactive protein (hs-CRP). IgA antibodies to MAA-LDL correlated with rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPA), fasting plasma glucose and IgA antibodies to *A. actinomycetemcomitans* and in IgA- and IgG-class with respective antibodies to *P. gingivalis.*

Conclusion. Among various arthritis groups, antibodies to MAA-LDL were most common in RA. Antibodies to modified lipoproteins associated with inflammation measured by ESR and hs-CRP. IgA antibodies to MAA-LDL correlated with age, antibodies to periodontal bacteria, RF, ACPA, and fasting glucose. Associations between antibodies to MAA-LDL and antibodies to periodontal bacteria, RA-associated antibodies, inflammatory parameters and plasma glucose reflect cardiovascular burden in inflammatory joint diseases already at diagnosis.

INTRODUCTION

Cardiovascular (CV) diseases and their risk factors were more common in patients with rheumatoid arthritis (RA), ankylosing spondylitis (AS), and psoriatic arthritis (PsA) than in matched controls in a US study conducted in 2000-2001 (1). At population level metabolic syndrome (MetS) has been identified as a risk factor for CV diseases (2). Oxidized low-density lipoprotein (oxLDL), which arises from lipid peroxidation of mostly polyunsaturated fatty acids within LDL particles, has been associated with atherosclerosis (reviewed in 3). Malondialdehyde (MDA) is one of the breakdown products of oxLDL. Antibodies to MDA-acetaldehyde adducts (MAA) have been recorded in atherosclerosis, alcoholic liver disease, and various inflammatory conditions including RA (3,4).

MDA-modified proteins have been identified in synovial tissue, and shared peptides between MDA-modified and citrullinated actin and vimentin have been detected (5,6). On the other hand, inflamed human gingival tissue provides an extrasynovial source of MAA, citrullinated and carbamylated proteins (7). Periodontitis is a chronic inflammation caused by gram-negative bacteria, for example *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* (8). Antibodies to periodontal pathogens can be measured from serum (9). Seropositive subjects can be considered pathogen carriers and the serum antibody levels associate with the corresponding bacterial amounts in saliva (10). IgA-class antibodies, with a short half-life, arise from a continuous exposure.

The aim of the present study was to assess the occurrence of antibodies to MAA-LDL and periodontal bacteria in patients with DMARD (disease-modifying anti-rheumatic drug)-naive inflammatory joint diseases.

PATIENTS AND METHODS

Patients. Data on all newly diagnosed patients with an inflammatory joint disease in the age-group ≥16 years were collected by the rheumatologists practicing in the Northern Savo rheumatological outpatient departments in an epidemiological prospective survey from 1st January to 31st December 2010 as described earlier (11). In this study RA was defined according to the American College of Rheumatology (ACR)/ European League against Rheumatism (Eular) 2010 classification criteria (12). Spondyloarthritis definitions have been described earlier (11). Patients with AS, PsA, reactive arthritis, arthritis with inflammatory bowel disease or unspecified spondyloarthritis (SpA) were included in the SpA group. As undifferentiated arthritis (UA) were classified cases with other rheumatoid factor (RF)- and/or anti-citrullinated protein antibody (ACPA)-positive mono- or oligoarthritis or RF- and ACPA-negative mono-, oligo-, or polyarthritis. MetS was defined according to the National Cholesterol Education Program (NCEP) panel ATPIII definition (2). MetS analysis was done for patients > 18 years of age.

Serum and plasma samples were stored at -70˚C. IgM-RF was evaluated by using immunoturbidimetry (Cat 20764574 322, Roche Diagnostics GmbH, Mannheim, Germany), and ACPA were identified by using EliA CCP (Phadia AB, Uppsala, Sweden). Values above 14 IU/mL and 7 U/mL were considered positive for RF and ACPA, respectively. Fasting plasma glucose was evaluated by enzymatic hexokinase method. Serum high sensitivity C-reactive protein (hs-CRP) was measured with particle enhanced immunoturbidimetric assay (Cat 04628918 190, Roche Diagnostics GmbH, Mannheim, Germany).

Serum IgA, IgG, and IgM antibody levels to MAA-LDL were determined using chemiluminescent immunoassay (13). Briefly, MAA-LDL was immobilized on 96-well white microtitre plates. Non-specific binding sites were blocked with 0.5% fish gelatin in 0.27mM PBS-EDTA. Serum samples (1:100 – 1:2000) were diluted in PBS-EDTA and incubated for 1 hour. The bound Ig was determined with appropriate alkaline-phosphatase-conjugated secondary antibodies and Lumi-Phos (Lumigen, MI) as substrate. Data are expressed as relative units (RU) determined from internal human Ig standard-curve.

Serum IgA- and IgG-class antibodies against periodontal bacteria *A. actinomycetemcomitans* and *P. gingivalis* were determined by multi-serotype ELISA (9). CV% 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 (EU) in IgA-class and ≥ 5 EU in IgG-class.

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

STATISTICS

Data are presented as means with standard deviations (SD), medians with interquartile ranges (IQR) or as counts with percentages. The statistical significance between groups was evaluated with the chi-square test, Mann-Whitney test, analysis of variance (ANOVA), and the Kruskall-Wallis test. Correlation coefficients were calculated by the Spearman method; Sidak adjustment was applied to correct the levels of significance for multiple testing. The normality of variables was evaluated graphically and using the Shapiro–Wilk W test. Stata statistical software, release 15.1 (StataCorp, College Station, Texas) was used for the analyses.

RESULTS

Demographic and clinical features of the 135 patients, 53 (39%) with RA, 44 (33%) with SpA and 38 (28%) with UA, are presented in Table 1. HLA-B27 was studied in all patients with AS and in 26/36 in the rest of the SpA group. Six of eight patients with AS (75%) and seven of 26 other SpA cases (27 %) were positive for the antigen. MetS was detected in 49%, 30%, and 47% of RA, SpA, and UA patients, respectively. Prevalence of IgA antibodies to *P. gingivalis* differed between the disease categories occurring in 53%, 21% and 41% of RA, SpA, and UA patients (p=0.005), respectively. Prevalence of IgA antibodies to *A. actinomycetemcomitans* was 89 % in RA, 77% in SpA, and 87% in UA, (p=0.28). IgA antibodies to *A. actinomycetemcomitans* correlated with RF with a Spearman coefficient (r)=0.29 (p=0.001) and IgA to *P. gingivalis* with ACPA with r=0.28 (p=0.002). Being edentate did not correlate with the antibody levels to periodontal bacteria.

The age- and gender-adjusted anti-MAA-LDL-IgA (p=0.009), -IgG (p=0.031) and -IgM (p=0.001) antibody levels differed between the diagnostic categories (Figure 1). The antibody levels were highest in RA. Anti-MAA-LDL levels did not differ between patients with MetS compared to those without in any diagnostic category. Correlations of antibody levels to MAA-LDL with patients' age, antibodies to periodontal bacteria, RF, ACPA, ESR, hs-CRP and fasting plasma glucose are shown in Table 2. Ever smoking, ever use of alcohol or being edentate did not associate with the antibodies to MAA-LDL.

DISCUSSION

In this sample of newly diagnosed patients with an inflammatory joint disease, we found that antibodies binding to MAA-LDL differed between the disease categories, but not in patients with MetS present compared to those without in early disease. Antibodies to MAA-LDL correlated with ESR, hs-CRP and fasting glucose. Anti-MAA-LDL-IgA levels also correlated with antibodies to periodontal pathogens and RA-associated autoantibodies.

Antibodies to MAA have been shown to associate with ESR in RA similarly to the present study (4). In an earlier study, the antibodies binding to MAA associated with RF and ACPA in patients with a mean RA disease duration of twelve years (12), whereas we report here that this correlation is present already at diagnosis. In a study on antibodies to MDA-adducts, the antibody levels in RA were highest in early disease and associated with inflammatory parameters and disease activity (5). IgG anti-MDA did not correlate with IgG ACPA, but IgA and IgM anti-MDA correlated with respective ACPA. IgG and IgM anti-MDA levels were increased in PsA patients compared to healthy controls, but the levels were lower than in patients with RA (5). In addition, the anti-MDA reactive antibody clones derived from human RA patients' synovial B or plasma cells displayed strong binding to human oxMDA-LDL.

Increased IgA to MAA-LDL, dependent on components of MetS, has been reported in patients with type 2 diabetes compared to subjects with normal glucose metabolism (13). Those groups that had the highest plasma IgA antibody titres to MAA-LDL had also the highest plasma concentrations of tumour necrosis factor-α or hs-CRP. In our series half of the patients with RA and UA and one third of the SpA patients had MetS. The anti-MAA-LDL-IgA levels were highest in RA, and they correlated with the fasting glucose.

Saliva IgA and IgG binding to oxLDL have shown cross-reactive properties with *P. gingivalis* (14). In the present study IgA antibodies to *P. gingivalis* showed significant association with anti-MAA-LDL-IgA and -IgG. Systemic antigenic challenge like teeth cleaning induce rapid toll-like receptor (TLR)2 expression on circulating B-cells (15). TLR2 ligands can also induce J chain and IgA expression by mucosal B-cells (15). The high prevalence of IgA antibodies to periodontal bacteria and IgA anti-MAA-LDL antibodies may arise from mucosal stimulus in the present study.

The limitations of the present study are the small sample size, cross-sectional nature of the study 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 (11). The study design did not include the control subjects. However, our patients were DMARD naive and the median symptom duration was 6-12 months.

The present study describes associations between antibodies to MAA-LDL, antibodies to periodontal bacteria, RA-associated antibodies, inflammatory parameters and plasma glucose in newly diagnosed arthritis patients. The data support the theory on the presence of cross-reactive epitopes in oxidized lipoproteins and periodontal bacteria. Molecular mimicry of these epitopes inducing mucosal immune responses possibly links arthritic, periodontal and CV diseases. High prevalence of MetS and occurrence of antibodies to lipid peroxidation products at diagnosis support the importance of evaluating and treating CV risk factors in all patients with arthritis right from diagnosis.

ACKNOWLEDGEMENTS

The authors thank Sirpa Rannikko for her expert technical assistance.

Compliance with Ethical Standards:

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (the Ethics Committee of the Kuopio University Hospital 127/2009) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Table 1. Demographic and clinical features and antibodies to periodontal bacteria in patients with rheumatoid arthritis (RA), spondyloarthritides (SpA) and undifferentiated arthritis (UA).

	RA N=53	SpA N=44	UA N=38	P-value between groups
Female, n (%)	26(49)	21 (48)	27 (71)	0.059
Age, mean (SD)	59 (11)	44 (15)	52 (14)	<0.001
BMI, mean (SD)	27.2 (5.0)	26.5 (4.5)	28.8 (6.0)	0.13
>30, n (%)	10 (19)	10 (24)	12 (33)	0.57
Duration of symptoms, median, IQR, months	7 (5,14)	12 (5,44)	7 (3,17)	0.094
ESR, mean (SD)	23.7 (22.1)	13.6 (19.3)	24.7 (29.4)	0.001
RF pos (%)*	41 (79)		3 (8)	<0.001
ACPA pos (%)	39 (74)		1 (3)	<0.001
General VAS	54 (26)	45 (23)	46 (26)	0.22
HAQ	0.71 (0.66)	0.38 (0.43)	0.59 (0.65)	0.060
DAS28(ESR) (SD)	4.3 (1.3)		3.5 (1.2)	0.002
BASFI (SD)		22.3 (20.2) 33†/44		
BASDAI (SD)		34.5 (22.2) 33†/44		
Smoking, n (%)				0.44
Never	25 (47)	16 (36)	16 (42)	
Ex-smokers	14 (26)	16 (36)	16 (42)	
Smoking	14 (26)	12 (27)	6 (16)	
Ever use of alcohol (%)	35 (69)	36 (88)	25 (68)	0.059
Metabolic syndrome, n (%; 95% CI)	26 (49; 36 to 62)	13 (30; 18 to 44)	18 (47; 32 to 63)	0.12
Edentate patients (%)	47 (89)	24 (56)	31 (86)	<0.001
AaIgA, mean (SD) (EU)‡	5.1 (3.1)	3.9 (2.4)	4.1 (2.1)	0.084
AaIgG, mean (SD) (EU)‡	3.3 (1.9)	4.0 (2.2)	4.4 (2.8)	0.077
PgIgA, mean (SD) (EU)‡	3.8 (3.7)	1.9 (2.1)	3.5 (4.2)	0.001§
PgIgG, mean (SD) (EU)‡	6.1 (4.9)	4.3 (2.4)	6.3 (4.8)	0.081

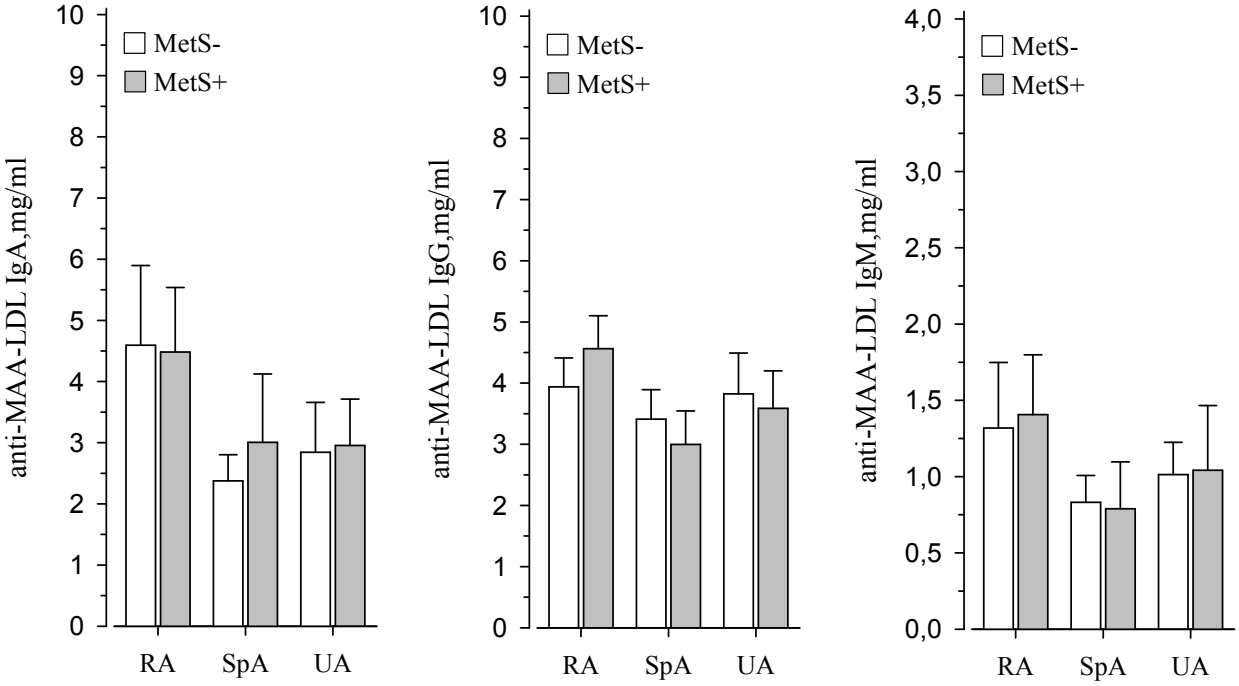
BMI= body mass index, ESR= erythrocyte sedimentation rate, RF= rheumatoid factor, ACPA= anti-citrullinated protein antibody, VAS =visual analogue scale, HAQ= Health assessment questionnaire, DAS= disease activity score, BASFI= Bath ankylosing spondylitis functional index, BASDAI= Bath ankylosing spondylitis disease activity index, Ig= immunoglobulin, Aa= *Aggregatibacter actinomycetemcomitans,* Pg= *Porphyromonas gingivalis,* EU= ELISA unit, *data available for 52 patients, †data available for patients with inflammatory back pain, ‡data available for 134 persons, §between RA and SpA groups

Table 2. Correlations of immunoglobulin A (IgA), IgG and IgM anti-MAA-LDL antibodies to age, antibodies to periodontal bacteria, rheumatoid arthritis-associated antibodies, inflammatory and metabolic markers in disease-modifying anti-rheumatic drug -naive patients with rheumatoid arthritis, spondyloarthritis and undifferentiated arthritis.

	Anti-MAA-LDL IgA	Anti-MAA-LDL IgG	Anti-MAA-LDL IgM
	Spearman (r)	Spearman (r)	Spearman (r)
Age	0.32**	0.23	0.01
AaIgA	0.42***	0.17	0.04
AaIgG	0.04	-0.00	-0.12
PgIgA	0.51***	0.25*	0.005
PgIgG	0.18	0.19	0.03
RF†	0.38***	0.15	0.09
ACPA‡	0.39***	0.23	0.05
ESR	0.33**	0.47***	0.30**
hs-CRP	0.31**	0.35***	0.24
fP-glucose	0.33**	0.14	-0.01

MAA-LDL=malondialdehyde-acetaldehyde modified low-density lipoprotein, Ig=immunoglobulin, Aa=*Aggregatibacter actinomycetemcomitans*, Pg=*Porphyromonas gingivalis*, RF= rheumatoid factor, ACPA= anti-citrullinated protein antibody, hs-CRP=high sensitivity C-reactive protein. Sidak was applied to correct the levels of significance for multiple testing p-values: *** $p<0.001$, ** $p<0.01$, * $p<0.05$. †n=118 ‡n=119

Figure 1. Serum immunoglobulin (Ig)A, IgG, and IgM antibody levels binding to malondialdehyde-acetaldehyde modified low-density lipoprotein (MAA-LDL) expressed as relative units (RU) determined from internal human Ig standard in patients with rheumatoid arthritis (RA) with metabolic syndrome (MetS) absent (n=27) or present (n=26), spondylarthritis (SpA) with MetS absent (n=31) or present (n=13) or undifferentiated arthritis (UA) with MetS absent (n=20) or present (n=18).



MAA-LDL = malondialdehyde-acetaldehyde adducts low-density lipoprotein, Ig =immunoglobulin, RU= relative unit