Effect of biological therapy on levels of atheroprotective antibodies against phosphorylcholine and apolipoproteins in rheumatoid arthritis – a one-year study

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Abstract

Objective

To examine how treatment of rheumatoid arthritis (RA) with anti-tumour necrosis factor alpha antagonists (anti-TNF) and B-cell targeting rituximab influences novel markers of atherosclerosis and inflammation, such as atheroprotective natural IgM antibodies against phosphorylcholine (anti-PC), oxidised low-density lipoprotein (oxLDL) and apolipoproteins.

Methods

In a prospective study 215 patients with RA were enrolled of whom 85.6% were seropositive, aged 57.9±12.4 years, with mean disease duration 8.5 (5–15) years. 162 patients were treated with anti-TNF and 53 with rituximab for one year. The patients were assessed and blood sampled at 0, 3, 6 and 12 months. IgM anti-PC and oxLDL were determined by ELISA and apolipoproteins by immunoturbidimetry.

Results

IgM anti-PC increased by 26% during anti-TNF treatment, $p<0.001$, while decreased by 14% on rituximab, $p=0.023$, after 12 months of treatment. Patients in remission after 12 months, DAS28<2.6, had higher baseline anti-PC levels compared with those not in remission in both anti-TNF, $p=0.007$, and rituximab-treated subjects, $p=0.041$.

In both treatment groups, levels of oxLDL increased temporarily at three months but apoA1 improved throughout the study. This effect was inversely correlated with changes in disease activity. The apoB and apoB/apoA1-ratio remained stable throughout the whole study period.

Conclusions

Anti-TNF treatment demonstrated a favourable long-term effect on anti-PC levels. Low levels of IgM anti-PC may identify immune-deficient state and predict inferior therapy response. Biological therapies increased the level of the anti-atherogenic lipid apoA1. The impact of these effects on future CVD events deserves further studies.

Key words

antibodies against phosphorylcholine, apolipoprotein A1, biological therapy, remission, rheumatoid arthritis, rituximab, tumour necrosis factor inhibitors

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects 0.5-1% of the general population (1). Cardiovascular disease (CVD) represents the major source of serious comorbidity and mortality and accounts for approximately 60% increased risk of all RA-related deaths (2). Recently it was suggested that RA may equal type 2 diabetes mellitus as an independent risk factor for CVD (3). Traditional Framingham risk factors and inflammation-associated factors may contribute to the increased risk of CVD in RA (4).

During recent years the treatment of RA has improved due to the introduction of biologics, including anti-tumour necrosis factor alpha (TNF) antagonists and B-cell-targeting rituximab. It has been reported that TNF-α blockade improves endothelial function in patients with RA (5). Several studies consistently indicate that anti-TNF treatment ameliorates the risk of CVD (6, 7). Still, the effect of TNF blockade on cardiovascular function is not clear (8, 9). In addition, dampening of inflammation in RA by TNF blockade has been shown to induce pro-atherogenic changes in lipid profiles (10), but data on short-term and long-term effects are inconsistent (11).

Rituximab inhibits B-cell function by targeting CD20, and is increasingly used in rheumatic and autoimmune diseases including RA, especially in treatment of patients who do not respond to anti-TNF-treatment (12). Little is known about the exact role of B cells in atherosclerosis, but both pro- and anti-atherogenic effects have been suggested (13-15). Recent studies indicate that short-term treatment with rituximab improves vascular function and dyslipidemia (16); moreover, it decreases pro-thrombotic biomarkers (17). Thus, anti-CD20 treatment may reduce future CVD risks in RA but the data are limited.

We have recently reported that low levels of IgM natural antibodies against phosphorylcholine (anti-PC) independently predict CVD (18-21) and that there is a negative association between anti-PC levels and development of human atherosclerosis (22). Further, low levels of IgM anti-PC were associated with systemic lupus erythematosus (SLE) and carotid plaque occurrence (23, 24). No studies devoted to anti-PC in RA and effects of anti-TNF treatment have, however, been published. Also the effects on anti-PC levels following B-cell depletion are still unknown.

The objective of this study was to explore whether treatment with biologics as TNF blockade, with etanercept, infliximab, adalimumab, and anti-CD20 therapy, with rituximab, during one year influenced IgM anti-PC, oxidised low-density lipoprotein (oxLDL) and the apolipoprotein profile.

Methods

Patients

Two hundred and fifteen outpatients with RA (25) were identified from a prospective cohort of patients registered in the local database, including all patients treated with biologics at the Rheumatology Department, Karolinska University Hospital Huddinge. They started their treatment with anti-TNF (etanercept, infliximab and adalimumab) or rituximab between January 2000 and October 2007 and were enrolled consecutively in this study if they had remained on the treatment for at least one year, moreover, in case of anti-TNF treatment if they had been biologic-naïve earlier. At that time rituximab was used mainly in patients that had failed on anti-TNF-therapy, and thus, 68% of these patients had received anti-TNF treatment earlier. Rituximab was the first biologic in patients with a history of recurrent infections, lymphoma, lung fibrosis or family history of multiple sclerosis. A single course of rituximab was given during the study period. Concomitant disease-modifying anti-rheumatic drugs (DMARDs) were chosen by the treating physicians in accordance with the current recommended treatment strategy in Sweden.

Disease activity was assessed at treatment initiation and after 3, 6 and 12 months. This included soluble C-reactive protein (CRP; mg/l) and erythrocyte sedimentation rate (ESR, mm/h) measured by routine methods, as well as a composite disease activity score based on evaluation of 28 joints,

DAS28. DAS28<2.6 was classified as disease remission (26). Functional disability was assessed using a Swedish version of Stanford Health Assessment Questionnaire, HAQ (27).

In addition, information was obtained about rheumatoid factor (RF) positivity, current smoking, body mass index (BMI; kg/m^2), hypertension, history of diabetes mellitus, and previous CVD (angina pectoris, myocardial infarction, ischaemic stroke and congestive heart failure), as well as current medications. Hypertension was defined as a blood pressure above 140/90 mm Hg and/or treatment with anti-hypertensive drugs. The information about diabetes mellitus, angina pectoris, previous myocardial infarction, ischaemic stroke and congestive heart failure was retrieved through patients' medical records with a physician's diagnosis.

The study was approved by the ethics committee at Karolinska Institute, Stockholm, Sweden, reference number 2008/159-31, and was performed in accordance with the Helsinki declaration and with the informed consent of the patients.

Serum analysis

Sera were obtained at baseline, 3, 6 and 12 months of follow-up and stored at -70˚C until all samples were analysed. Anti-PC IgM and oxLDL were determined by enzyme linked immunoassay (ELISA) commercial kit (Athera Biotechnologies AB, Stockholm, Sweden and Mercodia AB, Uppsala, Sweden, respectively) according to the protocols provided by the manufacturers and essentially as described earlier (19, 28).

IgM anti-PC and oxLDL levels were expressed as arbitrary units. Population-based reference for oxLDL (obtained from 149 ambulatory, randomly selected individuals in the Stockholm area, Sweden) is mean 61, range 26–117, U/l. No international references for anti-PC or oxLDL are available to date.

Apolipoproteins A1 (apoA1) and B (apoB) were determined by immunoturbidimetry (Modular Analytics P, Roche Diagnostics) at the study centre for laboratory medicine, Karolinska University Hospital, Stockholm, Sweden. The apoA1 reference intervals, given by the study centre, are 1.10–2.10 g/l for women and 1.10–1.80 g/l for men. The apoB reference interval is 0.50–1.50 g/l for individuals younger than 40 years and 0.50–1.70 g/l for those who are of 40 years and older.

Statistical analysis

Calculations were performed using STATISTICA, release 8 (Stat Soft Scandinavia AB, Tulsa, OK, USA). Clinical characteristics were compared between groups using Kruskal-Wallis test for multiple samples or Mann-Whitney U-test for two samples for continuous variables, chi-square test or Fisher's exact test for dichotomous variables. We employed McNemar's test for dichotomous outcomes in analysis of differences in one sample at different time-points. The association between baseline level of antibodies and progression of clinical characteristics was determined using Spearman rank correlation. To analyse longitudinal changes in IgM anti-PC we used repeated measures ANOVA in the complete dataset with probabilities for *post-hoc* test for between group comparisons at different time points. A multivariate approach was applied as indicated in order to elucidate the possible effect of intergroup differences. Log transformations were undertaken if needed in order to obtain normality of variable distributions. The assumptions for analysis were valid. Level of significance was chosen to be $\alpha<0.05$.

Results

Patients' demographic and clinical characteristics

Demographic and clinical characteristics of the patients studied are summarised in Table I.

The characteristics were similar for the etanercept, infliximab and adalimumab patients, apart from a lower proportion of hypertension (48%) in patients offered etanercept, $p=0.040$, and a higher proportion of concomitant use of methotrexate (MTX) (98%) among patients starting infliximab, $p<0.001$, the latter in conformity to treatment recommendation.

Patients started on rituximab were older, had longer disease duration, higher frequency of RF-positivity and occurrence of erosive joint disease than the anti-TNF treated patients. These patients were less commonly treated with MTX but instead more often with other DMARDs and with glucocorticoids (GC). Further, they had higher frequency of CVD comorbidity and treatment with statins.

Disease activity and function

All patients had high disease activity at baseline measured by CRP, ESR and DAS28, and low functional capacity measured by HAQ with no intra group difference for the anti-TNF agents. However, patients started on rituximab had significantly higher DAS28 and HAQ-score than the anti-TNF treated groups, Table I.

Already at the 3-month follow-up, about 2/3 of the patients in both treatment groups experienced significant improvement according to DAS28, thus, mean ±SD DAS28 in the total anti-TNF group and the rituximab group was 3.9±1.2 and 4.2±1.3, respectively. This positive effect was present during the whole follow-up period reaching a mean ±SD DAS28 3.7±1.5 *vs*. 4.5 ±1.6 at the end of the study. The reduction in DAS28 throughout the study period did not differ between anti-TNF and anti-B-cell treated subjects, $p=0.11$.

Physical ability improved during follow-up as well, thus, HAQ-score at 12 months had decreased, mean ±SD, 0.3±0.5 in anti-TNF treated patients and with 0.2±0.4 in rituximab-treated subjects, without significant intra group differences among anti-TNF agents or between anti-TNF group *vs*. rituximab group, $p>0.05$.

Concomitant treatment

The DMARD therapy was fairly constant during the study period. At baseline and at the 12-month check-up the rituximab group was more frequently treated with GC, compared to the anti-TNF treated group, $p<0.001$. The GC treatment was stopped in low percentages of the patients and similarly in both treatment groups. In both groups the use of NSAIDs diminished during the study period. Treatments for hypertension and hyperlipidemia were unchanged throughout the study.

Table I. Baseline demographic and clinical characteristics of 215 RA patients, by treatment group.

	Anti-TNF total n=162	Rituximab n=53	*p*-value
Age, yrs	56.2 ± 12.4	63.3 ± 10.7	**0.001**
Female, n (%)	117 (72.2)	45 (84.9)	0.06
Duration of RA, yrs	7 (4-14)	11 (7-17)	**0.003**
RF-positivity, n (%)	132 (81.5)	52 (98.1)	**0.003**
Erosive disease, n (%)	137 (84.6)	51 (96.2)	**0.026**
Current smoking, n (%)	39 (24.1)	9 (17.0)	0.32
Hypertension, n (%)	90 (61.2)	32 (60.4)	0.91
Diabetes mellitus, n (%)	9 (5.6)	7 (13.2)	0.07
CVD comorbidity, n (%)	46 (28.4)	25 (47.2)	**0.012**
Statin use, n (%)	6 (3.7)	6 (11.3)	**0.040**
BMI, kg/m²	25.2 ± 4.6	25.9 ± 4.5	0.33
CRP, mg/l	22 (10-41)	28 (14-57)	0.11
ESR, mm/hr	36 (26-55)	36 (28-65)	0.25
DAS28	5.7 ± 1.0	6.1 ± (1.1)	**0.010**
HAQ-score	1.3 ± 0.6	1.5 ± 0.5	**0.033**
MTX use, n (%)	126 (77.8)	23 (43.4)	**<0.001**
GC use, n (%)	54 (33.3)	42 (79.3)	**<0.001**
GC, mg/day	5 (5-7.5)	7.5 (5-15)	**0.001**
NSAID, n (%)	112 (69.1)	31 (58.5)	0.15

Values are denoted as mean ±SD or median (IQR) depending on values distribution.
p-values are given for differences between the anti-TNF total and B-cells therapy groups. Bold *p*-values are statistically significant.
CVD: cardiovascular disease; BMI: body mass index; DAS28: disease activity score for 28 joints; HAQ: health assessment questionnaire; DMARD: disease modifying anti-rheumatic drug; GC: glucocorticoid; NSAID: non-steroidal anti-inflammatory drug; yrs: years.

Levels of antibodies against phosphorylcholine (anti-PC) of IgM subclass

At baseline there was no statistically significant difference in anti-PC levels between the total anti-TNF group *vs*. the rituximab group, p=0.44 (Table II). The anti-PC levels at baseline were significantly lower in male compared with female in the whole cohort, median (IQR) 37.8 (26.9–65.8) *vs*. 55.6 (33.1–96.7) U/ml, p=0.007. Next, the anti-PC level was negatively correlated with age, R Spearman -0.25, p<0.001. We did not find significant influence of any other baseline variables on anti-PC levels. Thus, anti-PC levels did not differ in RF-positive and RF-negative patients, a corresponding median (IQR) 50.7 (32.4–92.5) and 42.4 (24.4–88.5), respectively, p=0.44.

In the total anti-TNF group, anti-PC had increased significantly already after 3 months of therapy, and a further increase was evident at both 6 and 12 months (Table II). There was also a trend towards increasing antibody levels after 3 months of treatment in all three anti-TNF groups, reaching statistical significance at 12 months in patients on etanercept and infliximab therapy. In contrast to anti-TNF treatment, in patients receiving rituximab there was a statistically significant decrease in anti-PC levels at 12 months (Table II).

At all time-points of follow up, the anti-PC levels were significantly higher in the total anti-TNF group *vs*. the rituximab-treated group as presented in Figure 1, adjusted for age and gender. The overall treatment effect on antibody levels was strikingly evident, p<0.001. To elucidate possible influence of other variables, we used multivariate repeated measures analyses in which together with age and gender we included one by one the variables that differed between treatment groups at baseline, p<0.05. The multivariate approach did not change the results, which proved that the difference in anti-PC development during treatment with anti-TNF and rituximab was independent of difference in baseline disease severity.

Table II. Levels of IgM anti-phosphorylcholine (anti-PC) antibodies at baseline and changes during the follow-up, by treatment group.

Anti-PC, U/ml	Etanercept	Infliximab	Adalimumab	Anti-TNF total	Rituximab
Baseline	67.61 (46.4-115.8)	41.59 (33.6-88.5)	36.59 (24.3-64.6)	51.61 (32.9-94.5)	42.88 (31.6-81.5)
Δ 3-0	6.87 (-4.54;26.83)	2.56 (-6.23;11.98)	0.07 (-1.91;8.56)	2.25 (-4.03;13.24)	-5.2 (-10.69;-1.64)
p-value	0.15	>0.5	>0.5	**0.012**	0.35
Δ 6-0	4.99 (-3.86;34.73)	6.46 (-2;12.3)	-0.85 (-4.08;7.1)	3.92 (-3.63;19.6)	-6.11 (-11.35;-0.35)
p-value	0.28	>0.5	>0.5	**0.001**	>0.5
Δ 12-0	8.67 (-5.82;42.6)	8.47 (-2.05;19.14)	4.73 (-1.99;15.23)	8.27 (-2.38;27.92)	-8.02 (-15.28;-1.14)
p-value	**0.002**	**0.046**	>0.5	**<0.001**	**0.023**

Results are indicated as median (IQR). Δ: changes; 3-0, 6-0 and 12-0 concern differences between respective periods of follow-up, 3, 6 and 12 months and 0: baseline.
p-values are given for changes in anti-PC between the respective time-points and are marked in bold if statistically significant <0.05.
The number of patients was in the etanercept group 60 at all time-points; in the infliximab group 60 at baseline, 58 at 3 months, 48 at 6 months and 58 at 12 months; in the adalimumab group 42 at baseline, 42 at 3 months, 28 at 6 months and 32 at 12 months; and for the rituximab group 53 at baseline, 50 at 3 months, 52 at 6 months and 48 at 12 months.

Association between IgM anti-PC levels and disease activity

At baseline, there were no significant correlations between anti-PC concentration and levels of the inflammatory markers, disease activity, functional score or lipid profile measured by apolipoproteins.

The baseline levels of anti-PC in the total anti-TNF group differed in subjects who reached remission at the end of the study, DAS28<2.6, compared with those not in remission. Thus, on anti-TNF treatment, patients achieving remission had significantly higher baseline anti-PC levels, p=0.007, and this difference in anti-PC levels was

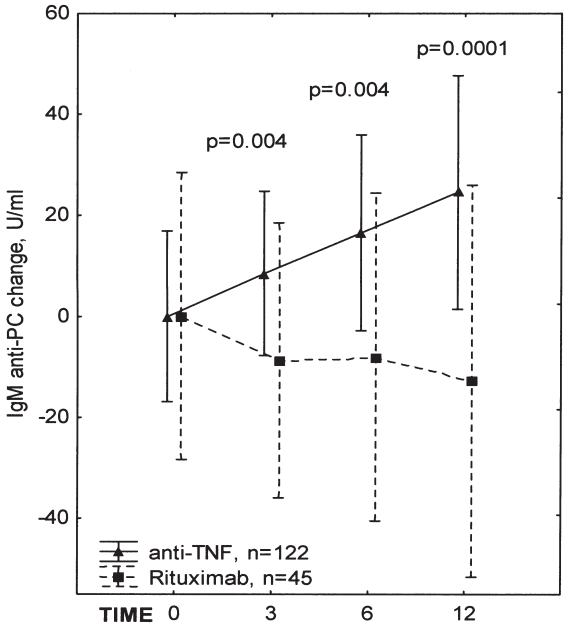

Fig. 1. Changes of IgM anti-PC levels from baseline over 3, 6 and 12 months of follow-up in the anti-TNF total and Rituximab groups. Adjusted (sex and age) LS means are showed where the vertical bars are 0.95 confidence interval. Post-hoc test (pooled MSE, between and within effects considered) was used for comparisons at different time points.

present at all follow-up time-points (Fig. 2A). Also, on rituximab, the baseline anti-PC levels were higher in those who later achieved remission, p=0.041 (Fig. 2B).

Levels of oxLDL and apolipoproteins

At baseline, oxLDL levels did not differ between the groups. At 3 months, oxLDL had increased significantly both on anti-TNF and rituximab treatments, on average, by 4% and 6% respectively, but this increase waned thereafter (Table III).

Baseline levels of apolipoproteins did not differ statistically between the treatment groups (Table III). The mean apoA1 was normal, >1.29g/l, but the mean apoB was elevated >0.88 g/l resulting in a high mean of pro-atherogenic apoB/apoA1 ratio >0.69 in all study groups.

ApoA1 levels increased throughout the study, at 3 months in the total anti-TNF group, at 6 months in the total anti-TNF and in the rituximab group, and towards 12 months apoA1 stabilised but maintained still higher levels than at baseline (Table III). Thus, at the end of follow-up apoA1 had increased, on average, by 8.5% on etanercept, by 2.3% in the anti-TNF total and by 6.7% on rituximab, with no significant inter group difference, p>0.05. The apoB and apoB/apoA1 ratio remained relatively stable throughout the whole study period and did not change significantly over time (Table III).

Association between lipid levels and disease activity

Associations between lipid changes and changes in inflammatory markers during the period of follow-up were similar in the patients on TNF-blockade and rituximab. In the whole study population absolute change (Δ) in oxLDL levels were inversely correlated with ΔCRP: at 3 months, r=-0.18, p=0.009; at 6 months, r=-0.26, p=0.001, and at 12 months, r=-0.24, p=0.001. In the same way, a low-moderate negative association was observed between ΔapoA1 and ΔCRP at 3 months, r=-0.17, p=0.108, at 6 months, r=-0.23, p=0.002, and at 12 months, r=-0.27, p=0.0001. We did not detect any significant associations between apoB, apoB/apoA1 ratio and changes in disease activity.

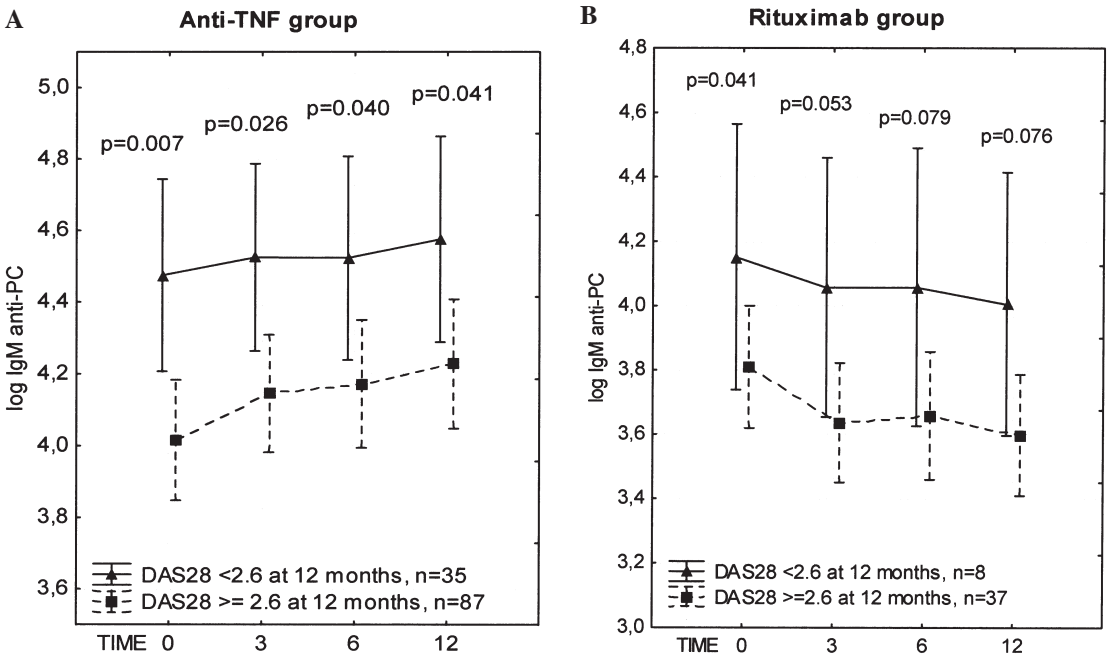

Fig. 2. Panels **A** and **B** represent anti-PC changes in responders *vs*. non-responders as to remission (DAS28<2.6) in anti-TNF total and rituximab groups under the follow-up. Original data log transformed, adjusted (sex and age) LS means where vertical bars denote 0.95 confidence interval. Post-hoc test (pooled MSE, between and within effects considered) was used for comparisons at different time points. DAS28 = disease activity score calculated on 28 joints.

Table III. Lipid levels at baseline and changes at the follow-up time-points, by treatment group.

Lipids	Etanercept	Infliximab	Adalimumab	Anti-TNF total	Rituximab
OxLDL, U/l					
Baseline	79.4 ±33.3	67.3 ± 19.7	57.9± 20.3	69.3±27.0	61.2 ±17.5
Δ 3-0	0.14 ±27.3	2.5 ± 12.8	5.9± 13.4	2.5±19.7	3.7 ±12.2
	p=0.75	*p*=0.10	***p*=0.004**	***p*=0.011**	***p*=0.023**
Δ 6-0	1.4 ±28.5	2.9 ± 17.5	6.6± 26.6	3.0±24.6	2.8 ±11.9
	p=0.54	*p*=0.26	*p*=0.88	*p*=0.20	*p*=0.10
Δ 12-0	1.6 ±29.4	-0.1 ± 13.9	-0.4± 18.1	0.6±22.1	2.1 ±14.6
	p=0.39	*p*=0.80	*p*=0.35	*p*=0.31	*p*=0.31
ApoA1, g/l					
Baseline	1.29 ±0.31	1.34 ± 0.30	1.28± 0.29	1.31±0.28	1.34 ±0.28
Δ 3-0	-0.02 ±0.32	0.05 ± 0.31	0.10± 0.31	0.04±0.32	0.03 ±0.25
	p=0.70	*p*=0.11	***p*=0.017**	***p*=0.013**	*p*=0.30
Δ 6-0	0.03 ±0.30	0.01 ± 0.33	0.02± 0.39	0.02±0.33	0.09 ±0.32
	***p*=0.037**	*p*=0.23	*p*=0.31	***p*=0.012**	***p*=0.022**
Δ 12-0	0.11 ±0.22	-0.03 ± 0.38	0.01± 0.47	0.03±0.36	0.09 ±0.32
	***p*=0.001**	*p*=0.87	*p*=0.53	***p*=0.007**	*p*=0.06
ApoB, g/l					
Baseline	0.88 ±0.22	0.90 ± 0.28	0.92± 0.28	0.90±0.26	0.95 ±0.25
Δ 3-0	-0.04 ±0.23	0.05 ± 0.22	0.07± 0.20	0.02±0.22	0 ±0.18
	p=0.46	*p*=0.09	***p*=0.042**	*p*=0.11	*p*=0.97
Δ 6-0	-0.01 ±0.20	-0.01 ± 0.21	-0.06± 0.23	-0.02±0.21	0.03 ±0.18
	p=0.77	*p*=0.80	*p*=0.20	*p*=0.51	*p*=0.19
Δ 12-0	0.04 ±0.17	-0.01 ± 0.25	-0.07± 0.36	-0.01±0.25	0.03 ±0.22
	p=0.12	*p*=0.78	*p*=0.48	*p*=0.47	*p*=0.67
B/A1 ratio					
Baseline	0.70 ±0.21	0.70 ± 0.24	0.74± 0.25	0.71±0.23	0.73 ±0.23
Δ 3-0	-0.02 ±0.15	0.01 ± 0.17	0.02± 0.18	0.003±0.16	-0.01 ±0.14
	p=0.50	*p*=0.60	*p*=0.64	*p*=0.95	*p*=0.78
Δ 6-0	-0.02 ±0.13	0.02 ± 0.24	-0.05± 0.20	-0.01±0.17	-0.02 ±0.15
	p=0.37	*p*=0.45	*p*=0.23	*p*=0.11	*p*=0.56
Δ 12-0	-0.03 ±0.11	0.02 ± 0.19	-0.06± 0.20	-0.02±0.17	-0.02 ±0.17
	p=0.12	*p*=0.57	*p*=0.22	*p*=0.31	*p*=0.25

Results are indicated as mean ± SD or median (IQR) depending on values distribution.
Δ: changes; 3-0, 6-0 and 12-0 concern differences between respective periods of follow-up, 3, 6 and 12 months and 0. baseline.
The number of patients as indicated in Table II.
p-values are given for changes in the lipids between the respective time-points and are marked in bold if statistically significant <0.05. There were no significant group differences between total anti-TNF *vs*. rituximab treatment.
OxLDL: oxidised low-density lipoprotein; apoA1: apolipoprotein A1; apoB: apolipoprotein B; B/A1: apoB/apoA1.

Discussion

One important finding in this report is that IgM antibodies against an epitope in the phospholipid moiety of membranes including oxLDL, anti-phosphorylcholine (anti-PC), increased during one year treatment of RA with TNF inhibitors in contrast to treatment with rituximab, a B-cell inhibitor. These observations may have several implications.

Anti-PC are natural antibodies being of major importance in the early response to lethal infections with PC-exposing Streptococcus pneumoniae, in mice (29). PC may play different roles in immune reactions, which are both protective and deleterious (30). Recent studies indicate that anti-PC, especially of IgM subclass, could play a role in atherogenesis and chronic inflammation. Thus, anti-PC may be atheroprotective in humans, since they are negatively associated with atherosclerosis development (22). Further, low anti-PC levels are independently associated with increased risk of CVD (18-21). In line with the clinical findings, mice experiments indicate that both active and passive immunisation raising anti-PC-levels lead to decreased atherosclerosis development (31, 32).

We have previously reported that individuals from Kitava, New Guinea, with a traditional life-style, have higher anti-PC levels compared to age- and sex-matched Swedish controls, and, interestingly, these individuals do not seem to suffer from CVD and only to a very limited degree of rheumatic diseases (33). Next, in a case-control study on patients with systemic lupus erythematosus (SLE) low IgM anti-PC were associated with SLE disease *per se* and also with SLE disease activity and severity, and notably, this low anti-PC was in contrast to the increase in other antibody levels (23).

Based on the findings summarised above, we have proposed a novel hypothesis that low levels of anti-PC represent an immune-deficient state associated with an increased risk of chronic inflammatory diseases, for example, atherosclerosis. Further, low anti-PC may promote disease activity and disease outbreaks in auto-inflammatory condition through its anti-inflammatory properties and potentially also through its anti-apoptotic function (34).

In this aspect, our finding that higher anti-PC levels were more common among individuals achieving remission in RA than among patients not in remission after one year of biologics use, though given the inherent limitation of post hoc analysis, raises the question whether the treatment with anti-PC could intensify other treatments and also have a positive effect *per se* in RA.

There are two potential mechanisms by which anti-PC could protect against cardiovascular disease. Firstly, anti-PC has anti-inflammatory effect through inhibition of inflammatory phospholipids of endothelial cells, such as platelet activating factor (PAF) (23). Another mechanism could be inhibition of macrophage uptake of oxLDL by anti-PC (20). These anti-inflammatory properties may have implications for chronic inflammatory diseases in general, not only CVD and/or atherosclerosis *per se* (35).

The mechanism by which anti-TNF treatment was associated with increased anti-PC levels in the present patients with RA is not clear. One possibility is that TNF-α has a direct inhibitory effect on B-cells producing anti-PC, but it is also possible that anti-PC is increased indirectly as a consequence of decreased inflammatory burden in general.

Growing evidence indicates that anti-TNF treatment can modify cardiovascular risk in RA (36). It is likely that a beneficial effect of anti-TNF treatment on IgM anti-PC may, at least partly, contributes to cardio-protective effects of this therapy.

While anti-TNF-treatment has been extensively studied in the context of CVD, little is known about the risk of CVD following rituximab treatment in rheumatic disease. Two small studies indicate that endothelial function may improve during such treatment (16, 37).

In humans, treatment with rituximab induces an almost complete depletion of circulating B-cells that usually lasts for 6 to 9 months, and in humans and primates persistent partial B-cell depletion may be found also in bone marrow and lymphoid organs (38). Theoretically, depletion of B cells in autoimmune diseases should be limited to conventional B2 cells while sparing regulatory B10 cells and potentially protective B1 cells, producers of natural antibodies (39), but this has not been proven in humans. It has been shown that some but not all antibodies decrease after B-cell depleting therapy, for example, levels of serum IgG, IgA and antibacterial antibody levels remain relatively steady, whereas a single course of rituximab therapy leads to fall in titres of RF, anti-citrullinated protein antibodies (ACPA), anti-dsDNA and modest decreases in serum IgM (40, 41).

The differential effect on anti-PC by the anti-TNF and rituximab treatments despite similar reductions in disease activity is challenging. The differences of patients' characteristics in the treatment groups, including use of methotrexate therapy, could be a source of confounding, but multivariate analysis controlled for this potential bias. As outlined above probable explanations for the diverse anti-PC courses are inhibition of anti-PC production by TNF-α, an effect that is hampered by anti-TNF, and decrease of B-cells anti-PC synthesis by rituximab.

Caution is warranted in interpreting data from immunoassays in the presence of RF in serum samples because of possible influence on results (42). Still, in our material we could not find any statistically significant difference in anti-PC levels between RF-positive and RF-negative patients. Further, the affinity of natural antibodies as IgM anti-PC to the antigen is much lower than for antibodies raised as an adaptive immune response. Also, it should be noticed that reduction in the levels of specific autoantibodies, such as RF, has been reported during anti-TNF (43) and B-cells therapy, nevertheless, anti-PC levels increased in the anti-TNF group in contrast to a decrease in antibody levels on rituximab in our study.

In the present study, both anti-TNF and rituximab treatment transiently worsened the oxLDL-status, but improved apoA1-status during the whole study period. It is likely that this corresponds with the trend of increased pro-atherogenic lipids, such as total cholesterol and triglycerides, but also increased anti-atherogenic lipids, such as high-density lipoproteins (HDL) and apoA1, in relation to decreased inflammatory measurements reported in other studies (44). In fact, the oxLDL and apoA1 levels were inversely correlated with changes in RA disease activity in the present patients, too. Consistent with earlier reports the magnitude of changes in apoA1 was fairly mild (11).

In RA, dyslipidemia combined with enhanced activity of pro-inflammatory cytokines leads to a pro-oxidative state, which further promotes oxidation of low-density lipoprotein (LDL) to the highly atherogenic oxidised LDL (ox-LDL). oxLDL has also pro-inflammatory effects such as activation of monocytes, endothelial cells, T-cells and B-cells (45, 46). The immune stimulatory and pro-thrombotic effects of ox-LDL appear to be mediated through the platelet activating factor (PAF) receptor where phosphorylcholine (PC) is the major ligand (4). Interestingly, oxLDL and foam cells are present in synovia in RA (47). Several studies in the general population reported a gradually increasing risk for CVD events with increasing plasma oxLDL (48, 49). To our knowledge, possible changes in circulating oxLDL during therapy with biologics have not been examined before.

In contrast to some studies but in line with others (44), in both anti-TNF and rituximab groups we determined a beneficial increase in apoA1 evident already after 3 months of follow-up, which lasted during the whole one year of treatment. At the same time, apoB and atherogenic index did not change significantly. Thus, the effect on lipids in our study was not agent specific but rather a result of reduced disease activity.

The limitations of our study are the small sample size of the separate treatment groups and that the study is of an observational character, thus, it is not based on a randomised design. Then, although the primary study interest was examination of potential CVD risk factors, information about incident CVD events throughout the study was lacking. At the same time, the strength of the study is in its prospective design, structured collection of blood samples and RA-disease activity measurements during the follow-up.

Active treatment of RA is required to reduce the risk of developing CVD events. Still, a number of interplaying pathogenic immune-inflammatory and genetic mechanisms should be considered in the development of atherosclerosis in RA. Long-term observational studies are needed to elucidate the potential role of biologics in the progression of cardio-vascular disease.

Conclusion

We here report that levels of the atheroprotective and anti-inflammatory anti-PC increased during one year of anti-TNF treatment in contrast to patients treated with rituximab. Furthermore, exploratory analyses showed that low IgM anti-PC levels predicted an inferior response to biological therapy. The results of the study extend previous observations that apoA1 improves but atherogenic index remains stable during long-term biological treatment.

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